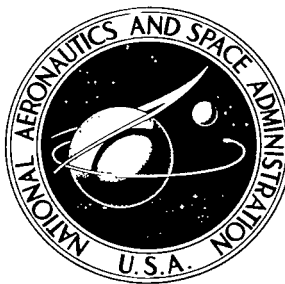


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**DEVELOPMENT OF RECEPTOR STRUCTURES
IN THE INNER EAR OF VERTEBRATES**

by L. K. Titova

"Nauka" Press, Leningrad, 1968

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EAR OF VERTEBRATES

By L. K. Titova

Translation of "Razvitiye retseptornykh struktur
vnutrennogo ukha pozvonochnykh"
"Nauka" Press, Leningrad, 1968

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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DEVELOPMENT OF RECEPTOR STRUCTURES IN THE INNER EAR OF VERTEBRATES,
L.K. Titova, "Nauka" Press, Leningrad, 1968, 192 pp.

ABSTRACT

In this book the author brings forth new, for the most part, still unpublished data on the embryonal development of receptor structures of the vestibular apparatus and of Corti's organ (the organ of hearing), obtained as a result of the application of an original method of isolation of a developing inner ear in representatives of five classes of vertebrates. The morphological and cytochemical changes which originate in the process of differentiation of the receptor structures of the inner ear (the vestibular portion of the labyrinth in the higher and lower vertebrates, the organ of hearing in birds and mammals) are compared.

In a sufficiently complete literary survey, data on the morphological, submicroscopic and cytochemical construction of the receptor structures of the inner ear of mammals and their change with adequate influences are selected. The nature of the chemical and ultrastructural changes in the process of differentiation of the organ and its definitive function is analyzed. 73 illustrations, 458 bibliographic citations.

Editor-in-Chief
Ya.A. Vinnikov

DEVELOPMENT OF RECEPTOR STRUCTURES IN THE INNER
EAR OF VERTEBRATES

L.K. Titova

INTRODUCTION

The inner ear of vertebrates arose in the phylogenetic process on the basis of the lateral line organs and originally preserved communication with the external environment. Such a labyrinth in a representative of the osteostraci had only two vertical semicircular canals (Stensiö, 1927). Among present species of animals, such a characteristic is present in the lamprey (Tret'yakov, 1916). Thus in the process of evolution, the complication of the lateral line organs and the functions connected with them led to the formation of a completely new sensory organ, i.e., the organ of vertebrate equilibrium, or the inner ear. The first change in the function of part of the lateral line organ took place on the organ of equilibrium. /3*

Soon there appears in the labyrinth system a third, horizontal semicircular canal (in the shark and skate). In the process of further evolution, the sacculus provides a number of supplementary formations, achieving in various classes of vertebrates, and sometimes even in individual orders (for example, the crocodiles), a different degree of development, i.e., the lagena and the basilar papilla (Lagena and Papilla basilaris), the fusion of which, in the higher reptiles and birds, first forms the cochlear canal (ductus cochlearis); in the latter a new receptor organ, i.e., Corti's organ, or the organ of hearing, is found. This organ is morphologically developed in the form of a straight cochlea in birds or a spiral cochlea in mammals. The lagena is preserved in monotremate mammals, but disappears in marsupials; in all the higher mammals, the cochlear canal is developed only on the basis of the Papilla basilaris.

Despite the migration of vertebrates onto the dry land, the labyrinth and the cochlea remained submerged in the internal environment of the organism. In addition, they seemingly preserve that fluid medium (in the form of endolymph and perilymph) in their open space, the wave oscillations of which specified the stimulations of the receptor structure of the lateral line. Thus another, the second, change in function took place. From the equilibrium organ one more morphophysiological formation was separated; i.e., the organ of hearing.

Thus, the inner ear of vertebrates represents a closed system of membranous sacs and tubules filled with endolymph, called the membranous labyrinth. It consists of two sacs: a round one (the sacculus) and an oval one (the utricle), and the three semicircular canals opening into the latter, which are situated in three

* Numbers in the margin indicate pagination in the foreign text.

mutually perpendicular planes. Each semicircular canal forms an expansion at its anterior end, i.e., the ampulla, which is directly connected with the utricle.

In all the enumerated sections of the membranous labyrinth (the inner ear) there are receptor organs, which include three types: auditory spots, i.e., maculae (located in the sacculus, utricle and lagena); auditory crests, i.e., the cristae (located in the ampullas of the semicircular canals); and the so-called perilymphatic organs, i.e., the Papilla basilaris of higher reptiles, birds and mammals, in which it bears the name "Corti's organ". Anatomically, /4 all these formations are differentiated both in their form and construction, as well as chiefly by the structure of their covering formations. Thus for the auditory spots, i.e., the maculae, the presence of an otolith membrane of a gelatinous substance and one large (fish) or many small otoliths (otoconia) sealed in it, is characteristic; for the auditory crests, i.e., cristae, the presence of the so-called cupula is characteristic, which is a gelatinous formation penetrated by many vertical canaliculi, the base of which is located at the summit of the crista; for the perilymphatic organ the presence of the so-called tectorial membrane is characteristic, which is a thin membranous covering formation, having unilateral attachment and a mobile basilar membrane.

At the basis of the organs of the acoustical labyrinth system of all vertebrates lie the so-called secondary sensory receptor cells which first appeared in primitive agnathous fish (Agnatha) in the Silurian period (Stensiö, 1927; Bystrov, 1957) in the system of the lateral line organs. At the present time the latter formation is present in fish and amphibians and was specialized for the perception of vibrations of the entire infralow frequency spectrum arising in the water. Despite the complex evolution of the lateral line organs, on the basis of its new derivatives, i.e., the organs of equilibrium and hearing, its original receptor form is preserved as before, i.e., the secondary sensory receptor hair cells. It is necessary to note that in the present labyrinth, its numerous functions are in no way as strictly delimited from one another as was earlier supposed.

The numerous functions of the inner ear with a comparatively monotonous morphological organization of its individual receptor organs lead to a consideration of the fine structural and chiefly chemical differences between individual receptor organs and their receptor cells. In an attempt to answer this question, one immediately sees the insufficiencies in the investigation of this organ. Everything that we know about the inner ear is based either on old data of an anatomo-microscopic nature, the majority of which do not satisfy contemporary problems of investigation or rely on exceptionally interesting, but one-sided data on the electron microscopy of this organ. In recent years a number of interesting attempts at the unification of electrophysiological and electron microscopy investigations were made. However, even in this exclusively prospective

direction, problems of the chemism of receptor cells and the connection of chemical processes taking place in them, with the specific functions of the receptor organ, are completely ignored.

In addition, the absence of a uniform approach to the investigation of receptor structures of the inner ear with regard to the unity of their structure, genesis and evolution is particularly striking. In this regard, the investigation of the embryogenesis of receptor structures of the inner ear in representatives of all classes of vertebrates, by contemporary methods of histochemistry and electron microscopy, in combination with the usual morphological methods, is particularly valuable. A very insignificant number of studies, basically made at the end of the last and the beginning of the present century, were dedicated to studying the embryogenesis of the inner ear of vertebrates. However, such an investigation at the present time makes sense only in the case where the study of embryogenesis of receptor formations of the inner ear will be continued by the same methods, and in definitive stages both in a state of relative rest, and under conditions of adequate stimulation. Only such an investigation allows the possibility of combining the structure and chemism of various receptor cells, having indicated in what fashion they are united at the time of cell differentiation and how they define its function.

The monograph which we wrote with Prof. Ya.A. Vinnikov, devoted to the investigation of the structure and cytochemical organization of Corti's organ of mammals and the change of this organization while functioning is closer to such a statement of the problem. In addition, it was shown that the specific function of the auditory receptor cells is accompanied not only by structural shifts, but also by a change in concentration for activity of a number of chemical, biologically active substances, such as nucleic acids, proteins, functional groups of protein molecules, glycogens, seceyno-dehydrogenase, cytochrome oxidase, and the specific enzyme of neural conduction: cholinesterase. Only after obtaining these data was it possible to raise the question of the appearance of cytochemical organization and its connection with the differentiation of cellular structures during embryogenesis of Corti's organ in mammals, as well as of other receptor structures of the inner ear in all vertebrates. We conducted an investigation of the cytochemical organization of the receptor epithelium of the utricular macula in definitive forms with the participation of our laboratory staff, in which the receptor structures of the inner ear of vertebrates were investigated both in a state of relative rest and with adequate stimulation. Conducting all of the studies was made possible after the development of intravital isolation of the inner ear (cochlear and vestibular portions) of mammals as well as of all vertebrates, which was accomplished by Prof. Vinnikov and myself. /5

relying upon the use and development of a method of isolating the inner ear of representatives from all classes of vertebrates, both in a definitive and in the embryonal stages, in the present

study the task was set of investigating the development of the structural and cytochemical organization of the inner ear in specific representatives of various classes of vertebrates with the aid of histochemical and some electron microscopic methods, and simultaneously comparing them with the investigation of definitive stages and functional shifts in receptor structures of the utricle and Corti's organ.

In short, the question is raised of tracing the significance and behavior of a number of a chemical, biologically active substances and the structures connected with them in embryogenesis, for the support of a definitive structure even during its functioning.

This investigation may serve as a step towards the understanding of the basic regularities in the development of the structure and function of receptor formations of the inner ear on the cellular and subcellular levels of their organization, and to show those characteristics of chemism and structural organization which determine the evolution of the function (Orbeli, 1958) of the inner ear in various directions. Simultaneously, such an investigation provides materials for understanding the nature and localization of exchange processes in the receptor structures of the inner ear and the directions of their changes with a specific function, which signifies the prospect of regulating them by means of active interference.

THE STRUCTURE OF THE INNER EAR IN DEFINITIVE FORMS

The Vestibular Apparatus

The inner ear of the higher vertebrates, including mammals, consists of the equilibrium organ or, as it is called, the vestibular apparatus, and the organ of hearing, i.e., Corti's organ, located in the so-called cochlea. The entire preliminary history of the development of the inner ear indicates that besides the common source or origin, the unification of these two organs into one system may be explained by the similarity of the mechanism of stimulation and by the necessity for precise spatial adjustment of the ear for determining direction and location of a sound source. /6

The organ of equilibrium, or the vestibular apparatus, (the vestibulus of the labyrinth) consists of three semicircular canals located in different, mutually perpendicular, planes and of two small membranous sacs: an oval one (utricle) and a round one (sacculus). The function of the semicircular canals is to react to change in angular accelerations, and the function of the sacs is to react to change in linear accelerations (Goltz, 1870) or to a change in the position of the animal in the gravitational field, and also in vibrational activities. In the lower vertebrates which lack a cochlea (fish and amphibians), the sacs evidently also assume the role of the auditory receptor (Buddenbrock, 1952; Gernandt, 1959; Prosser and Brown, 1962).

The terminal organs of the vestibulus have much in common with one another, since they have a common origin from the common membrane of all terminal organs, i.e., the so-called Macula communis. In addition, topographically there are distinguished: receptor structures of the ampullas, i.e., the ampullar cristae or the auditory crests (Crista ampularis), located in the expanded portions, i.e., the ampullas of the semicircular canals connected with the base of the utricle; receptor structures of the auditory spot of the utricle, i.e., utricular macula (Macula utriculi), located at the base of the utricle; the Macula neglecta (Crista quarta of the old authors), located on the medial wall of the utricle; the sacculus--the saccular macula--Macula sacculi and lagena--lagena macula--Macula lagena.

The auditory spot or macula represents a receptor formation lined with a sensing or receptor epithelial layer, covered above by the gelatinous layer of the otolith membrane, on which are located either one large otolith (bony fishes) or small crystals (otoconia), sealed in the gelatinous otolith membrane (selachians, amphibians, reptiles, birds and mammals). The auditory crest, or crista, is lined with the same receptor epithelium as the macula, but differs from the macula primarily by the fact that above the surface of this receptor layer in the crista is located the soft gelatinous cupula. /7 Besides the auditory spots and crests, in the inner ear of vertebrates still another type of receptor formation, i.e., the auditory

papilla, is located, an example of which is the organ of hearing of the higher reptiles, birds and mammals. Their covering formations are extremely variable and often have a nature which is hard to determine, for which, however, a unilateral attachment around the receptor epithelium is characteristic; the tectorial membrane of Corti's organ may serve as an example of this. Each receptor or terminal organ usually functions as a unit. Only Corti's organ is an exception in this regard, in which, besides the possibility of its usual function, there are also individual functional regions (de Burlet, 1934; Vinnikov and Titova, 1961). It is also possible that even the utricular macula is anatomically and functionally subdivided into separate regions (de Burlet, 1934; Werner, 1940, 1960; Flock, 1964). Receptor (terminal) organs of the vestibule greatly resemble each other. Their receptor epithelium rests on a cushion of connective tissue containing nerve fibers, which in turn lies on the lining of the osseous wall of the labyrinth or, in the case of the utricular macula, on a thick lamina of dense connective tissue. The terminal organs are differentiated by the form of distribution of receptor epithelium, by the form and structure of the mantle layer and by the specific nature of the excitation of sensory hairs (Denker and Kahler, 1926-1927).

THE SENSORY SPOTS--MACULAE

(According to Data of Light and Electron Microscopy)

In mammals the receptor spot of the utricular macula (Macula utriculi) lies almost horizontally, and the receptor spot of the saccular macula (Macula sacculi) is situated almost vertically, so that in relation to each other, they form almost a right angle. On the basis of this fact, the hypothesis was formed that only the plane of the macula and its position in space have physiological significance and that the receptor epithelial layer of both maculae are completely similar and do not have any morphological peculiarities in their structure and distribution (Magnus, 1924; de Burlet, et al.). Starting from this hypothesis, de Burlet and Haas (de Burlet and Haas, 1923; de Burlet, 1930) constructed models of the plane distribution of auditory spots for the guinea pig, monkey (Macacus) and man. However, the investigation of the highly developed otolith apparatus of bony fish and birds (Werner, 1928, 1938a, 1938b) showed very significant differences in the structure of the epithelium, otolith membrane, otolith, marginal epithelium and innervation between both maculae. In mammals these differences are less pronounced but are completely evident (Werner, 1940). Moreover, both the utricular and the saccular macula are each located in three planes, which in turn indicates that each of them is an independent organ bearing its own specific function.

The utricular macula is located along the bottom, forward and medial walls of the utriculus. The basic portion, lying on the bottom, forms a 140° angle with the side portions. When the head is in the normal position, the basic portion of the utricular macula with

the otolith lying above the hair cells occupies an approximately horizontal position. The receptor epithelium has the form of an oval spot measuring 3 X 1, 5 X 2 mm and rests on the slightly concave basal membrane, which due to its formlessness is easily distinguished from the adjacent intumescence of connective tissue, penetrated by blood vessels. In man the utricular macula is elevated from the flat epithelium by a narrow transition layer of indifferent epithelium and turns into a high (0.036-0.04 mm) single-layered cylindrical epithelium, which consists of thread-like support cells and sensory hair cells (Denker and Kahler, 1926-1927). /8

Werner (1960), studying the utricular macula of fishes, noted that it consists of two clearly distinguished parts: one, the Cotillus, occupies the concave portion of the bottom of the utriculus, and the other, the Rampa, the marginal stria, forms the raised forward and lateral margin. The Rampa consists of a higher epithelium than the Cotillus, and is characterized by a thicker otolith membrane (Ramparium) with a surface which is not covered or only partly covered by otoliths. The Cotillus, on the other hand, is covered with otoliths and is connected with them by means of fibers which penetrate the inner surface of the otolith (Cotillarium). The Rampa turns into the same high surface of the macula without a sharp border. Between the Rampa and Cotillus the bottom of the utriculus is located a narrow stria, the striola, 4-8 receptor cells in width. The otolith membrane which is located above the striola is swollen. The nuclei of the supporter cells are located in the striola only basally, but the large nuclei of its sensory cells are in one layer (Werner, 1940). In the mouse, guinea pig and rabbit, the border between the Rampa and Cotillus is often distinguished by a narrow stria, the striola, with a swollen otolith membrane and a special type of epithelium, which was mentioned above. According to Werner's data (Werner, 1940), above the Rampa is located a high layer of large crystals, while the remaining surface is covered with a medium thickness of a layer of small crystals.

The saccular macula lies on the medial wall of the sacculus almost in a vertical position and forms an angle of around 90° with the utricular macula. In mammals the saccular macula is smaller than the utricular macula and has an oval shape. Its base lies directly on the vestibular osseous wall.

De Burlet (1931) found that in mammals (except for the echidna) the anterior portion of the saccular macula is bent laterally at an angle of approximately 160° and has a dorsal section which half turns and forms with an angle of 150° with the rest of the sacculus, and in the middle an angle of 140° (Magnus, 1924). Voit (1907) and van Oört (1919) mention that the dorsal section is innervated by special nerve bundles proceeding from the Ramus anterior N. octavus, while the nerve bundle innervating the base part is connected with the Ramus posterior. They think that the dorsal section has another function than the base part and is functionally connected with the utricular macula. De Burlet (1931) considers the double innervation

of the saccular macula to be the result of ontogenetic segmentation, depending upon the division of the Macula communis and the distribution of the sacculus in relation to other parts.

According to Werner's data (Werner, 1960), the same striola exists in the saccular macula even with the same characteristics as in the utricular macula. In the sacculus of the rabbit, the striola passes through the center of the surface, in the guinea pig near the inferior and anterior margin. The sensory epithelium of the saccular macula was of various heights. In the region of the striola it is around 18 μ . There is an especially high epithelium on the dorsal section and at the posterior end of the base part. Dorsal of the sensory epithelium lies a very high surface, covered with specific marginal epithelium, on which the otolith membrane is attached with the aid of strong marginal fibers (Werner, 1960).

While the utricular and saccular macula are present in all vertebrates without exception, the lagenar macula is absent in many vertebrates. In mammals it is present only in the monotremata. Its /9 spatial orientation is usually the same as that of the saccular macula. Only in Sauropsida is it located in the general chamber with the papilla acoustica. With the gradual development of this acoustic formation, the lagenar macula occupies less and less of the peripheral position, and in crocodiles and birds it is finally located at the end of the cochlear canal. It has the same distribution even in the echidna, but here it is strongly deflected from the basic direction of the cochlear canal.

It is characteristic of the macular lagena that it lacks a basic plane. On the whole, its structure is bent; often it is even shortened in the form of a spiral. The orientation of this surface in the space has not been precisely established at the present time. It is necessary to note that this sensory formation is well developed in both the sauropsida which are terrestrial and those which live in the air, and suddenly disappears in mammals (de Burlet, 1934). De Burlet considers that this disappearance is connected with the appearance of curls in the cochlea of mammals, due to which the end of the cochlea loses its firm spatial orientation.

The single-layered cylindrical receptor epithelium of any macula produces the impression of a multi-layered epithelium, since the receptor cells are located in its superior portion and their bases do not extend to the basal membrane. The supporting cells surrounding them have a long extended body which stretches from the basal membrane to the surface of the epithelium. Their nuclei lie at various heights, which creates the impression of being multi-layered. If we consider the surface of the epithelium, the macula upwards, then it is possible to see the free end of the receptor cells bearing sensory cells on their surface and the free ends of support cells located between them. Both these types of cells form a mosaic, whereupon each receptor cell is separated from the neighboring cell by the support cells surrounding it. Sensory hairs of receptor

cells of the macula have, according to Retzius' data, a length of 20-25 μ . Each cell is not a simple formation but consists of a certain number of extremely thin hairs glued to one another. These hairs are shorter on the periphery than on the center (Retzius, 1881, 1884; Kolmer, 1927; de Burlet, 1934). Moreover, on each cellular surface there is one whip or flagellum (Kolmer, 1927; Held, 1926), which de Burlet considered to be unsubstantiated for a majority of forms (de Burlet, 1934). At the present time the presence of such flagellum or cilium (kinocilia) with the characteristic structure is completely proven by the data of electron microscopy (Wersäll, 1956; Engström and Wersäll, 1958a, 1958b; Engström, 1960a; Vinnikov, Govardovskiy and Osipova, 1965a, 1965b et al.). The basal and the sensory cells are surrounded by the endings of nerve fibers. The nuclei of the sensory cells usually are round; those of the supporting cells are oval and contain 1-3 large nucleoli. At the apical end of the support cells there is usually a diplosome (Kolmer, 1927; Held, 1926; de Burlet, 1934). Held found intercellular supporting threads inside the support cells, analogous to those which are present in supporting cells of Corti's organ. The latter have the form of fibrils, which proceed from the basal membrane upwards and disperse there palmately, forming a network of locking laminae surrounding the hair cells. This network covers the entire surface of the macula with a cuticular membrane, pierced by openings into which the heads of the cells are inserted (Membrana reticularis).

The hair or receptor cells have a cylindrical or pitcher shaped form with a round base. In the lower part is located a nucleus which is rich in chromatin. Individual hair cells have a not entirely uniform length, and their nuclei are frequently located in two layers (Eckert-Möbius, 1926; Werner, 1940). In the protoplasm there are mitochondria, Golgi's apparatus and granular inclusions of fat and lipoids (Kolmer, 1927). Proebsting (1924) established a constant number of receptor cells characteristic of each type for each terminal organ in amphibians, while the number of supporting cells increases with the size of the body. The macula is always a zone of cells which takes part in the attachment of the otolith or the otolith membrane. An inner and outer region are usually distinguished in this zone. /10

THE AUDITORY CRESTS--CRISTAE (Crista ampularis)

The crista is located inside the expansion of the semicircular canal, i.e., the ampulla, from which it received the name ampullar crista (Crista ampularis). Corresponding to the three semicircular canals, an interior vertical, a posterior vertical and a lateral (horizontal) crista are distinguished. The crista is usually located perpendicular to the plane of its semicircular canal. The horizontal crista, which is situated almost parallel to this plane, is an exception. The crista itself may be either deeper or more sloping. The slopes of the crista are differentiated as the utricular and canicular, depending on whether they are turned to the utricular cavity or to the corresponding semicircular canal. In birds a fur-

ther complication in the form of the crista takes place. On both vertical cristae near the highest point of the crest a crest-like elevation is formed, located perpendicular to the main axis of the crista, which is called the Septum cruciatum. This formation, however, does not increase the area of receptor epithelium since the latter is absent on it (Retzius, 1884; de Burlet, 1934; Portmann, 1961). Portmann considers this structure to be a special adaptation to flight, attesting to the important role of the vertical semicircular canals for birds. It is absent in the horizontal crista, which preserves the form of a simple bend.

The receptor epithelium of the crista has various distribution. In one case it may cover both slopes of the crista as well as its summit; i.e., in this case the entire crista bears receptor epithelium upon it. In another case the receptor epithelium is located only on the middle portion of the crista. Between these two extreme forms there are transitional forms.

In many animals a subdivision of receptor epithelium of the vertical cristae is observed corresponding to the segmentation of the Ramus ampularis. In the horizontal crista there is no such subdivision of the receptor epithelium.

In mammals, the receptor epithelium of the cristae is not usually subdivided, but continuously covers the entire ridge of the crista, including both slopes. The bat (Iwata, 1924) and the cat (Retzius, 1884) are an exception in this regard, in the cristae of which there is a subdivision of sensory epithelium.

The height of the crista is usually equal to $1/3$ the diameter of the ampulla. Each end of the crista is enclosed in a crescent-shaped zone covered by supporting epithelium (Wersäll, 1956), i.e., the crescent-shaped lamina (Planum semilunatum), which was first described by Steifsand (1835). In the ampullas of both vertical canals this formation is located equally on both sides of the crista, but in the horizontal ampulla it is more developed in the superior wall of the ampulla.

Many authors (Retzius, 1884; Ramon and Cajal, 1909; Held, 1926; Kolmer, 1927; Lorente de No, 1931; Tello, 1931, et al.) studied the structure of the receptor epithelium of the crista under a light microscope. According to their data, the epithelium of each ampullar crista consists of a two-layered receptor epithelium, which is surrounded by a layer of cylindrical epithelial cells of transitional epithelium (Kolmer, 1927) along the periphery of the crest. The receptor and supportive cells, located on a well-developed basal membrane, are clearly distinguished from one another. Supporting cells pass through the entire thickness of the epithelial layer from its basal membrane to the apical surface, in contrast to the receptor cells which terminate in the upper third of the epithelial layer and their bases do not reach the basal membrane. In contrast to the other authors, Kolmer (1927) counts three types of cells in the sen-

/11

sory epithelium of the crista. According to Kolmer's description, the high portion of the crest is covered with receptor epithelium which consists of sensory and supporting cells, similar to those of the macula. And here the oval nuclei of the support cells are located in the base of the epithelium and the nuclei of receptor cells, which are larger and rounder, are closer to the apical surface. They are differentiated from receptor cells of the macula by the fact that their apical surface, covered with a cuticle, bears sensory hairs, each such hair being a bundle of very thin hairs. The marginal surface of each such hair cell forms a swollen cuticular ring, which is united with the terminal laminae of neighboring supporting cells with the aid of locking laminae (Kolmer, 1927). Altogether they form the network of reticular membranes, in the openings of which were located the heads of receptor and supporting cells (Wersäll, 1956). Nerve fibers innervating the hair cells pass in the crack-like space between the receptor and supportive cells. In the cytoplasm of supporting cells, Kolmer describes the poorly-visible supportive fibrils which do not reach the base of the cell. In the cytoplasm is found Golgi's reticular apparatus. The receptor epithelium covering the cristae of various ampullas differs in height. It is surrounded by a third type of cylindrical cells, the so-called Kolmer's transition epithelium, which in man is characterized by the unequal distribution of the nuclei, and usual type of cytoplasm.

Lorente de No (1931) distinguished three types of nerve endings in the crista of the mouse, which are distributed along various regions of the sensory epithelium. Together with Poljak he showed the same thing even for the human crista. Thick fibers innervate the central region of the crista. After penetrating the epithelium they branch very little and form calyces at their ends, which cover the bases of the hair cells. Each fiber may form not more than 2-4 calyces on a number of horizontal hair cells. The calyces formed by one fiber are usually connected with each other by collaterals. In addition the summit of the crista is distinguished by the separate cellular group located on it united by internal innervation. Lorente de No sees the appearance of a special differentiation of innervation in this fact; moreover, he attempts to find a regularity in the distribution of similar groups. Poljak (1927) considers that such a means of distribution serves for the condensation and intensification of innervation. Average fibers are distributed in the lateral regions of the crista. They even form scyphoid nerve endings and intra-epithelial plexus. These fibers are extremely branching and their calyces cover cells which lie far from one another so that an intersection of innervated regions takes place.

Thin nerve fibers are subdivided in the basal region of the crista. They do not provide scyphoid endings, only a very delicate intra-epithelial plexus, the nerve fibers of which form a thin net on the hair cells. Poljak (1927) connects the double innervation of the crista with the animal's ability to orient itself in various positions.

In the fresh state, the cupula located above the receptor epithelium of the crista is completely transparent and, due to the large water content on fixed preparations, appears as a very wrinkled formation. With a good fixation it has a striated structure in longitudinal sections. In cross section there are numerous thin openings in which sensory hairs are located, visible in section as fine points lying inside the openings. De Burlet (1931), Hensen (1878) and then Bowen (1932) consider that the cupula is formed with fixation as the result of the thickening of the endolymph. However, Hasse (1869), with intravital observations, established that the cupula occupies all the space between the sensory epithelium and the roof of the ampulla. This same thing was later confirmed by Steinhausen (1933, 1934) and Werner (1932). The fact that the structure of the cupula changes with fixation and decalcination was shown by Stotte (1928).

Wittmaack (1918), on the basis of his microscopic operations, considered the cupula to be immobile. Steinhausen (1934, 1935, 1937) showed experimentally that its function was connected with changes in position. He visualized the crista with the ampulla in the form of a hinge. He considered the subcupular space to be the housing of the hinge. According to his observations, the uniform curvature of the ampullar roof ensures their steady contact and covering of endolymph with bending of the cupula. Steinhausen (1935, 1937) concludes from the position of the lateral ampulla in the skull that the cupula is deflected not only by the endolymph current in the semicircular canal but also with the aid of its own inertia.

STRUCTURE OF THE VESTIBULAR RECEPTOR EPITHELIUM ACCORDING TO DATA OF ELECTRON MICROSCOPY

According to Wersäll's (1954) electron microscopic investigations, there are two types of hair receptor cells in the receptor epithelium of the vestibular apparatus of mammals both in the cristae and in the maculae, which he designated as Type I and Type II cells. A Type I receptor cell has a pitcher-like shape with a short thin neck and an expanded base in which the nucleus is located. This body is surrounded by characteristic nerve endings in the form of calyces which surround the cell and go almost to the cuticle. Type II cells usually have a cylindrical shape; however, great variation in form and size is observed in them. On the round base of these cells there are small bud-shaped nerve endings. The summit of the receptor cells of both types is provided with a large number of structureless macrovilli (stereocilia), among which, in each cell, there is one real flagellum, i.e., the kinocilium with a characteristic fibrillar apparatus consisting of 9 pairs of peripheral and 2 central single fibers. This fibrillar structure is characteristic for all true flagelli in the entire plant and animal kingdoms (Fawcett and Porter, 1954).

Type I Hair Cells

Each hair cell is surrounded exteriorly with a clear plasmatic membrane and is separated from the surrounding cells and nerve endings by an intracellular fissure 150-200 Å in width. The apical portion of the cells terminates in the cuticular lamina from which 50-80 stereocilia proceed. Among them, as was already mentioned, there is one hair with a typical fibrillar structure, the kinocilium. Each stereocilium has an extracellular and intracellular portion. The basal portion of the stereocilium consists of axial fibers surrounded by a thin margin of cytoplasm; the latter is exteriorly surrounded by a thin surface membrane, appearing as an extension of the plasmatic membrane of the cell. The axial fibril enters inside the stereocilium only 4 μ from the cellular surface. The upper part of a stereocilium lacks axial fibrils and is less elastic than the basal portion. At the base of a stereocilium, the axial fibril directly passes inside the cuticle, forming the intracellular portion of the stereocilium, and is surrounded by a light zone. /13

The kinocilium in each cell has a typical structure (Engström, 1951; Engström and Wersäll, 1952; Fawcett and Porter, 1954, et al.). As in the stereocilium, in the kinocilium there is distinguished an extracellular portion, the cilia proper, and an intracellular portion, the basal corpuscle. The extracellular portion consists of a central bundle of fibrils, surrounded by a thin cytoplasm. The kinocilium is covered with a thin external membrane, appearing as the continuation of the plasmatic membrane of the cell. The fibrillar bundle consists of two central single fibrils, surrounded by 9 pairs of peripheral fibers. The fibrils of the kinocilium directly enter the cytoplasm of the cell and there form the basal corpuscle.

Located at the summit of the receptor cells, the cuticle has a thickness from 0.2 to 0.5 μ and consists of a dark amorphous basic substance (matrix) and a large number of electron dense particles.

The cytoplasm of the cells contains a large number of different organoids, intracellular membranous systems (α - and γ -cisterns) and mitochondria. The α -cisterns (Sjöstrand, 1956; Wersäll, 1956) have 1-8 fissures, located in the subnuclear zone. Each such fissure is bounded by a membrane 40 to 60 Å in thickness. Exteriorly these membranes are sprinkled with ribosome granules 150-200 Å in diameter. The γ -cisterns are delimited by nongranulated, smooth membranes, forming a complicated system located in the supranuclear zone. These membranes evidently form Golgi's apparatus.

The mitochondria are clustered in three different regions. The fundamental group of round mitochondria is located directly below the cuticle. The diameter of these mitochondria is from 0.3-0.4 μ . A subsequent group of mitochondria is located around Golgi's apparatus. As a rule these are elongated mitochondria having a length of 0.8 μ . A third group of mitochondria are found in the subnuclear portion of the cell. Engström and Wersäll (1958b), starting from

the fact that mitochondria are often found in regions with a high metabolic level, expressed a hypothesis about the mosaic spatial distribution of definite functions in these receptor cells.

Golgi's apparatus is located in various zones of the supranuclear part of the receptor cell and, for the most part, is located in a region near the nucleus. It consists of a system of opaque membranes of high electron density bordering thin, canyon-like fissures, which often terminate in vacuolae of various sizes. The distance between individual membranes in each zone is generally constant. The smooth membranes entering into the composition of Golgi's apparatus do not have granules on them. In various zones on the periphery of Golgi's apparatus is observed a various number of irregular vasicular formations having a diameter of 200-300 Å. The basic substance surrounding the membrane of Golgi's apparatus is somewhat more dense than in other portions of the cytoplasm, but turns into it without a sharp boundary.

The nucleus of a receptor cell, for the most part, has a round shape with a diameter of around 8 μ . It is surrounded by a double membrane about 50 Å thick with a distance between each of them of around 150 Å. There are pores in the membrane. Inside the nucleus is located an opaque, homogeneous, randomly distributed mass in which small particles are scattered, combining into lumps or rods and a continuous layer of varying thickness extending from the inner side to the nuclear membrane. The nucleolus is a large dark body having the same structure as the rest of the dark mass of the nucleus. The inner space of the nucleus is filled with an amorphous light substance. /14

As electron microscopic investigations showed, Type I receptor cells are included in the very large scyphoid nerve endings, the presence of which was first described by Wersäll, (1956) in the crista of the guinea pig and by Pietrantoni and Iurato (1960) in the inner ear of man. However the scyphoid nerve endings were first described with the aid of the light microscope by Kaiser (1891) and Niemack (1893), although their data were refuted in the later investigation of Ramon and Cajal (1909) and Lorento de No (1926), who considered that the scyphoid nerve endings consist of a dense plexus of thin nerve fibers. However, contemporary electron microscope investigations affirmed the correctness of the observations of Kaiser and Niemack.

According to electron microscope data (Wersäll, 1956, 1960; Engström and Wersäll, 1958a, 1958b, 1958c, et al.), the scyphoid nerve endings almost completely surround the entire body of a Type I receptor cell, leaving free only the apical portion of the neck. Between the external plasmatic membrane of a hair cell and the membrane of the scyphoid nerve ending there is a distinct fissure 150-200 Å in width. The internal membrane of a scyphoid nerve ending is around 50 Å thick, and without interruption turns into the exterior marginal membrane of the nerve cup and then into the axial membrane

of the nerve fiber. The neuroplasm of the nerve cup is characterized by a very low electron density and contains small round mitochondria. Further along the axon, the mitochondria acquire a rod-like form and are very long and bent. In the apical portions of the nerve cup there are small synaptic vesicles (Wersäll, 1956; Smith, 1956). In its more distal portions there is located a small number of round granular or vesicular structures, earlier described in the presynaptic neuroplasm. Scattered granules can be easily found lower along the axon far from the nerve cup. Engström and Wersäll (1958a, 1958b) show that the fibrillar structures are always found in the axon but are not found in the nerve cup. However, in his 1950 survey on the innervation of vestibular hair cells, Wersäll mentions that in its proximal portion the nerve cup contains neural fibrils and mitochondria. From the external side of the nerve cup or nerve ramulus, forming a nerve cup, there is constantly found a system of supplementary granulated nerve endings, forming synaptic contact with the scyphoid nerve endings (Engström, 1958). They represent the so-called axonal nerve endings (Gray, 1962). With the high resolving power of an electron microscope it is possible to see the structure of the synapse, both between the hair cell and the scyphoid nerve ending and between the latter and the granular nerve ending lying on it. The presynaptic and postsynaptic membranes are separated by a clear synaptic fissure. Each plasmatic membrane consists of two osmophile layers divided by a nonosmophile fissure, so /15 that the entire formation is three layered (Smith and Sjöstrand, 1961). Type I hair cells are present only in reptiles (Vinnikov and Titova, 1962), mammals (Wersäll, 1956), and birds, (Vinnikov, Govardovskiy and Osipova, 1956b). They are absent in cyclostomata (Löwenstein, 1965), fish and amphibians (Löwenstein, et al., 1964; Flock, 1964; Vinnikov, 1964, 1965).

Thus Type I receptor cells in the inner ear of vertebrates, in the process of evolution, appear only after the position of the body of the animal changes in the gravitational field connected with the exit of animals onto the dry land and the transition to the terrestrial form of life. In other words the change in the construction of the receptor cells and its synaptic apparatus is connected with the complication of the function of the vestibular apparatus of vertebrates.

Type II Hair Cells

Type II hair cells have a more cylindrical body shape, a singular cuticular surface and a round or sharply sharpened base. The shape of their cellular body varies slightly in different receptor formations of the vestibular apparatus. They are innervated by numerous thin nerve fibers, terminating on their base in bud-shaped nerve endings. The cytoplasm of these cells contains a large number of equally distributed particles of moderate electron density. Some α -cisterns are found in an insignificant number, and agranular membranes, forming Golgi's apparatus, are regularly observed in their subnuclear zone. Isolated accumulations of mitochondria are

not observed, they are equally distributed along the cytoplasm of the cell. The cuticle and sensory hairs of Type II cells are identical to those already described for Type I hair cells.

Thus the most essential difference between Type II receptor cells and Type I cells, with the exception of the distribution of organoids in their cytoplasm, is in their innervation. As opposed to the large scyphoid nerve endings of Type I hair cells, the Type II cells are provided with a certain number of small bud-shaped nerve endings. Among them, Wersäll (1956) found two types of nerve endings; one containing a large number of synaptic vesicles or granules, and a second with an insignificant number or even complete absence of them. These are the so-called granulated and agranulated nerve endings. Both of these types of nerve endings are found in synaptic contact with receptor cells.

Insofar as in the lower vertebrates there are only cylindrical Type II cells with bud-shaped nerve cells in the receptor organs of the inner ear, while the receptor epithelium of the vestibular apparatus of the inner ear in higher vertebrates is characterized by the presence of pitcher-shaped Type I cells with scyphoid nerve endings aside from Type II cells, it seems to us that it would be more correct to call the cylindrical cells with the bud-shaped nerve endings Type I cells, and the pitcher-shaped cells with scyphoid nerve endings (which appear only in the higher vertebrates) Type II cells. However, in accordance with the fact that the given classification of receptor cells is firmly entered into the literature, with the aim of avoiding unnecessary confusion, we will subsequently maintain the generally accepted classification of these receptor cells proposed in due course by Wersäll (Wersäll, 1956).

Comparing both types of vestibular receptor cells, Wersäll (1956) indicates that Type I hair cells are highly differentiated cells with a more specific function than Type II cells. Each thick nerve fiber forms only several nerve cups located in a small region while the thin nerve fibers often innervate a large group of receptor cells, sometimes located at great distances along the entire epithelium. From these facts Wersäll concludes that Type II receptor hair cells react to stronger stimuli distributed along the surface of the entire epithelium, while Type I receptor cells are more sensitive and react with action on a more limited region of epithelium. Insofar as Type I hair cells are absent in the inner ear of lower vertebrates, they are evidently not absolutely necessary for the basic function of the vestibular apparatus.

/16

According to the data of Engström and Wersäll (1958a), supporting cells in the utricle of the guinea pig occupy the space between the receptor cells. Their diameter varies in different epithelial layers and their nuclei basically are located in the basal part of the cell. The nuclei have very clearly pronounced nucleoli and very densely distributed granules of chromatin. The body of the supporting cell stretches from the basilar membrane to the surface

of the receptor epithelium and contains a large number of cytoplasmic granules. The authors indicate that they did not observe systems of supporting fibers in them corresponding to the well-developed supporting fibrils of supporting elements of Corti's organ. The cytoplasm of the supporting cells of the utricular macula contain a well developed Golgi's apparatus, mitochondria and very osmophilic inclusions, which sometimes reached a significant size.

CYTOCHEMICAL CHARACTERISTICS OF THE VESTIBULAR RECEPTOR EPITHELIUM

Comparative electron microscope investigation of receptor structures of the vestibule of the labyrinth in vertebrates are practically absent. In a short report, Löwenstein indicates that in cyclostomata (larva of the lamprey) in the utricle there are only receptor cells of Type II provided with a bundle of stereocilia with one kinocilium. There are more detailed data on selachians (Löwenstein, et al., 1964). From this study, it follows that receptor cells in the labyrinth of the skate are located near the distal surface of the epithelium and never reach the basal membrane. These receptor cells are of only one type and in their superficial form and size are similar to Type II receptor cells of the vestibular apparatus of mammals (Wersäll, 1956; Engström and Wersäll, 1958a, 1958b). They differ by their dense cytoplasm. On the apical ends of a receptor cell a cuticle is located which is from 1-1.5 μ thick. From it proceeds a bundle of 20-70 stereocilia and 1 kinocilium with a characteristic fibrillar structure. These stereocilia reach a length of 40 μ in the crista and 5-10 μ in the macula. All the stereocilia in the bundle have an equal diameter of 0.2 μ . However, at the same time there are receptor cells with diameter of stereocilia of 0.1 μ . They are distributed in the form of the letter V, in the notch of which the kinocilium is located; i.e., their distribution is similar to the distribution of sensory hairs in surface hair (auditory) cells (Corti's organ in mammals) and is different from the distribution of sensory hairs on the vestibular cells of the remaining vertebrates. In the cytoplasm of a receptor cell are many round and rod-shaped mitochondria. In the supranuclear part of a cell is a large number of narrow fissures and vacuoli, surrounded by the membranes of Golgi's apparatus. Here there is a corpuscle with a dense central granular structure, surrounded by a single membrane. Other corpuscles contain a certain number of small vesicles. In individual receptor cells in the subnuclear portion are horizontal fissures parallelly distributed surrounded by a single membrane, from the surface side of which is located a layer of dense granules around 150 Å in diameter. This formation corresponds with that which was described in the vestibular Type I receptor cells in mammals (Wersäll, 1956). Vacuoles with a diameter of 300-700 Å are scattered in the light cytoplasm. /17

Supporting cells in the maculae and cristae of selachians are represented by column-shaped elements with an expanded basal end, in which an oval nucleus is located. Formations of supporting cells

are always located on the basal membrane. The contracted upper part of the supporting cell passes between receptor cells and reaches the surface of the epithelium. There are 3 supporting fibrils which are united at their distal ends into a ring of dense substance, located directly under the plasmatic membrane, and together they form a reticular membrane, inside which are located the summits of the receptor cells. The cytoplasm of a supporting cell contains a large number of vacuoli of a diameter of around 0.6μ and a certain number of granules in the supranuclear portions of the cells. There is an insignificant number of thin rod-shaped mitochondria. The cytoplasm is dense and contains a large number of small granules around $150-200 \text{ \AA}$ in diameter. Membranes of Golgi's apparatus are located vertically in the apical part of the cell. Löwenstein, Osborn and Wer-säll (Löwenstein, et al., 1964) described numerous microvili and a short cilium, which they called the ciliar rod, at the summit of the supporting cell. The ciliar rod has a structure characteristic for kinocilia but which does not exceed 0.5μ in length. Under it is a basal corpuscle with a basal peduncle. In contrast to kinocilia, here there is a second basal corpuscle located perpendicular to the axis of the first.

In individual receptor organs of the inner ear there is always the specific nature of topographic and functional polarization of the receptor cells. Thus, in the ampullar crista the kinocilium is always positioned in such a way that the axis passing through the sequentially located bundles is perpendicular to the long axis of the crista: in the crista of the horizontal canal, all kinocilia lie on the side of the bundle directed toward the utricle, in the cristae of the anterior and posterior vertical canal, the kinocilia always lie on the side of the bundle turned toward the canal. In the sac-cular macula of the skate, the kinocilia are located at a right angle to the long axis of the macula; in the dorsal half of the receptor epithelium, the kinocilia are turned backwards, and in the ventricle part ventrally or dorsally. Thus groups of cells from both directions of polarization alternate with one another. In the lacinia there is the same alternation of cell groups, but the kinocilia, in addition, are directed laterally or medially. In the Macula neglecta the kinocilium is located in all directions but always is turned to the periphery of the macula.

All nerve fibers in the inner ear of the skate are myelinated and the majority of the receptor cells located in the central part of the crista and utricular macula are each innervated by one single thin nerve ramulus; the remaining are innervated by several nerve ramuli. All nerve endings are surrounded by the plasmatic membrane separated from the membrane of the receptor cell by a synaptic fissure $100-200 \text{ \AA}$ in width. Granular and nongranular nerve endings are distinguished. The first contains synaptic, densely distributed vesicles and some mitochondria; the second, neurofibrils, mitochondria and some synaptic vesicles. The authors describe yet a third type of very small nerve endings, the nature of which is unclear.

/18

Histochemical investigations of receptor structures of the inner ear of selachians are completely absent in the literature available to us.

The structure of the utricle of bony fishes was studied on pike in our laboratory by Vinnikov, Govardovskiy and Osipova (1965a), and in the burbot by Flock (1964), under an electron microscope.

According to the data of Vinnikov, Govardovskiy and Osipova, the receptor epithelium of the utricle of a pike, as in other vertebrates, consists of receptor and supporting cells. Receptor cells have an elongated cylindrical shape slightly narrowing toward the top and a somewhat expanded, sometimes scalloped-out base at which nerve fibers arrive. The body of a receptor cell is bordered by a well-defined plasmatic membrane. A cuticle is located on the apical surface of the cell, which is differentiated by its very high electron density. In each receptor cell, from the cuticle proceed bundles of sensory hairs consisting of 8-15 stereocilia and one kinocilium which begins immediately in the cytoplasm of the cells. It is located strictly in a polar direction to one side of the hair cell. Stereocilia in the kinocilium are covered with a distinct double membrane which is a continuation of the plasmatic membrane of the cell. The kinocilium always has a clearly pronounced fibrillar skeleton, which proceeds from the basal corpuscle located in the cytoplasm of the cell. The axial fiber of a stereocilium passes inside the cuticle and then at a certain angle enters the cytoplasm. Under the cuticle are located mitochondria having an elongated oval, rod-like or corkscrew shape. These mitochondria are distinguished by a significant electron density of the superficial membrane and by the tube-like crista thickly filling the body of the mitochondria. The cytoplasm of the receptor cells is filled with large and small granules. There are free ribosomes. Golgi's apparatus are poorly pronounced. There are many vesicles of various sizes, the number of which sharply increases in the region of the synapses. The nucleus of a receptor cell is covered with a double plasmatic membrane with distinct pores and knot-like thickenings. Chromatin of the nucleus is distinguished by moderate electron density. It is equally distributed along the entire karyoplasm, in places forming a more pronounced thickening. There is a small nucleolus, distinguished by a great electron density. The bases of the supporting cells are located on the basilar membrane. They pass between the hair cells to the surface of the receptor layer. The cytoplasm of supporting cells has a significantly higher electron density than the cytoplasm of receptor cells. Cells are separated from one another by a well pronounced plasmatic membrane. On the apical surface of supporting cells, the plasmatic membrane forms microvilli. The nucleus of the supporting cell has an irregular oval shape elongated along the long axis of the cell and is located in the lower part of the body of the support. Its karyoplasm is filled with small, equally scattered, grainy chromatin. The nucleolus is poorly pronounced. In the cytoplasm small round mitochondria are more or less equally distributed having membranes and cristae which are of great electron density. They are

located chiefly in the apical or basal portions of the cytoplasm. Nearer to the receptor cells in the cytoplasm is located the neuroplasmatic network with ribosomes. There is also a large number of small and large granules and vacuoli. The basal membrane consists of two equal membranes which are pierced with myelinated nerve fibers entering from the connective tissue into the epithelium. Their axoplasma, within the limits of the receptor epithelium, is rich in mitochondria in the form of small grains with a paramural distribution of cristae. In addition to the myelinated nerve fibers, non-medullated nerve fibers penetrate into the receptor epithelium. At the base of the receptor cells, double nerve fibers are formed which are granulated and nongranulated. The authors indicate that individual nerve endings containing single large mitochondria may enter into synaptic contact simultaneously with two neighboring receptor cells. In this study there is also a detailed description of a structure of a synapse.

/19

Flock (1964), in his work on the investigation of the structure of the utricular macula in the herring, pays attention primarily to the structure and distribution of kinocilia due to which, in his opinion, the morphological polarization of the cell takes place, which is closely connected with its physiological polarization. The picture of ultrafine structure of receptor and supporting cells of the utricular macula in the burbot given by Flock in general differs little from that in the utricular macula of the pike. The basic difference is in the reticular lamina, which is well-developed in the pike due to a desmosome, and in the intense folding of the nuclear membrane of a reticular cell. We will not deal here with his detailed data on the characteristics of distribution of kinocilia in the utricular macula.

The equal distribution and relatively weak concentration of RNA in the cytoplasm is histochemically characteristic of the receptor elements of the inner ear in bony fishes. At the same time these cells are characterized by a high total concentration of protein, which is notably increased at the apical ends of the cells. The concentration of corpuscle groups of protein molecules in these receptor cells is quite low but their distribution differs in the very large selectivity and differentiation of their appearance in individual cellular structures. Thiol groups in these cells, as a rule, have a low concentration and equal distribution.

For neurons of the eighth ganglion in bony fishes, a higher concentration of RNA protein and functional groups of protein molecules is characteristic. With a very low concentration of DNA in the chromatin, their nuclei are characterized by a very high protein content and functional groups of protein molecules. The concentration of these substances is highest in the nucleolus.

Both for fresh water and for salt water bony fishes, the very high activity of specific cholinesterase (ACE) both in the region of synapsis in the maculae and cristae and in the neurons of the eighth

ganglion, in which an ACE is located not only in the region of synapsis but also intracellularly in the granules of Nissl's substance and in the nucleolus. The activity of respiratory enzymes of succinate dehydrogenase (SDH) and also of alcohol-, lactico- and malicodehydrogenase (ADH, LDH, MDH), which are located in the mitochondria of receptor cells and in nerve fibers adjacent to them, is also high. Lacticodehydrogenase is an exception in this regard in that it is also located outside of the mitochondria.

For receptor organs of the labyrinth of the frog, polymorphism of the receptor epithelium is more characteristic. The component receptor cells belong to Type II, for which, as was already noted, a cylindrical body forming innervation with the aid of bud-shaped nerve endings is characteristic. They are provided with one kinocilia, located polarly for each bundle of stereocilia. However for receptor cells of the labyrinth of frogs, a very great variability in cell body form is characteristic, with the preservation of innervation by means of bud-shaped nerve endings. Histochemically these receptor cells are also somewhat different from Type II receptor cells of bony fishes. Thus for receptor cells of the inner ear in the frog, a moderate concentration of RNA in the cytoplasm is characteristic, with an increase in RNA toward the apical end of the cell and the nucleolus. The same moderate concentration of DNA is observed in the chromatin of the nucleus. For these cells a moderate concentration of total proteins and carboxyl groups is also characteristic in the cytoplasm, and a high concentration of total protein in carboxyl groups is present in the nucleus, increasing the concentration of protein in cytoplasm in the perinuclear region. In contrast to bony fishes, in the receptor cells of the frog a very high concentration of the thiol groups is noted. /20

For neurons of the eighth ganglion of the frog, a moderate concentration of DNA and RNA is characteristic, with a sharp increase in the latter in granules of Nissl's substance located on the periphery of the cellular body. Protein and functional groups of protein molecules in the neurons of the eighth ganglion of the frog are characterized by moderate concentration and equal distribution.

Enzyme activity of ACE in receptor structures of the labyrinth of the frog and in the neurons of its eighth ganglion is more moderate, although the location of this enzyme and receptor structures of the inner ear in bony fishes and in the frog corresponds completely which undoubtedly attests to the similarity of their function (Titova and Vinnikov, 1964).

The activity of succinate dehydrogenase, alcohol-, lactico- and malicodehydrogenase in the inner ear of the frog is somewhat lower than in the receptor structures of the inner ear of fishes. They are located in large rod-shaped mitochondria of the receptor cells and nerve endings.

The receptor epithelium of the labyrinth of the tortoise is

interesting by the fact that instead of one type of receptor cell, as was the case in fishes and amphibia, here there are two types of receptor cells: cylindrical with bud-shaped nerve endings and pitcher-shaped receptor cells, the entire body of which is surrounded by a large nerve cup formed by nerve fibers approaching the cell. Just as in fishes and in amphibia, Type II and Type I cells are provided with one kinocilia located polarly in relation to the bundle (40-50) of stereocilia (Vinnikov, Gazenko, Titova, Bronstein, et al., 1965).

For all receptor cells of the inner ear of the tortoise, a mean concentration of nucleic acid in their nuclei and in the cytoplasm with an equal distribution of RNA in the latter is characteristic. A very intensive reaction to RNA is observed in the nucleolus. These cells are significantly richer in protein, especially the pitcher-shaped calls (Type I cells), in which a low concentration of protein is observed, even in the scyphoid nerve endings, in which RNA is completely absent. The protein content in nucleolus is unusually high. For receptor cells of the labyrinth of the tortoise, an eccentric location of the nucleolus is characteristic. The emergence of nucleolic RNA into the cytoplasm is even seen in individual receptor cells. For the inner ear of the tortoise a very high concentration of carboxyl groups in the cytoplasm of its receptor cells is characteristic, and an even greater concentration of the thiol groups is also characteristic. The latter especially distinguishes these receptor cells in the cytochemical characteristics of receptor cells of the inner ear in other vertebrates (Titova, 1966).

Enzyme activity of ACE in the inner ear of the tortoise, in /21 comparison with bony fishes, is more moderate, but it is higher than in the frog. However, the location of the enzyme in them completely corresponds. The collinergic mechanism for transmission of the nerve impulse, which appeared in various stages of evolution, is preserved even in the new, although still poorly developed receptor structure, i.e. Papilla basilaris, which appears in reptiles. This structure is connected with the auditory function, as is assumed by Titova and Vinnikov (1964). For receptor structures of the inner ear in the tortoise, an unusually high enzyme activity of respiratory enzymes (FDH, ADH, LDH and MDH) is characteristic not only in mitochondria located in the body of the receptor cell, but even in the nerve fibers ascending to them and especially in the scyphoid synapsis.

As was mentioned above, the separation of a special, well-developed section for the perception of acoustic frequencies, i.e. the Papilla acustica basilaris, which is located in a special outgrowth of the saccula (the cochlear canal), is more characteristic for the inner ear of birds. This organ (Corti's organ) is characterized not only by the presence of the covering formation, the tectorial membrane, but also by a special type of cylindrical receptor cells connected with bud-shaped nerve endings. In receptor cells of Corti's organ in birds there is one kinocilium located polarly

in relation to the stereocilia bundle, attached to the tectorial membrane (Held, 1926; Vinnikov, Govardovskiy and Osipova, 1965d).

According to the data of an electron microscope investigation carried out in our laboratory (Vinnikov, Govardovskiy and Osipova, 1965b), the utricle of the pigeon, as well as the utricle of other higher vertebrates, consists of Type I and Type II receptor hair cells with supporting cells at their endings. The receptor epithelium is separated from the underlying connective tissue by a broad (0.2 μ) and slightly twisted basal membrane. From the apical surface of both Type I and Type II receptor cells proceed 50-55 nonmobile stereocilia and one mobile kinocilium with the usual fibrillar structure ($9 \times 2 + 2$). The diameter of the stereocilia of the pigeon equals 0.3 μ . The cytoplasm of the stereocilia is filled with numerous thin fibers. The authors could not succeed in tracing a connection between the stereocilia and the otolith membrane.

A kinocilium is always located on one specific side of the hair cell under a portion of the cellular surface free from the cuticle. The distal end of a kinocilium, in the author's opinion, is not connected with the otolith membrane.

The cytoplasm of receptor cells contains a large number of mitochondria, some of which form an accumulation under the cuticle, closely adjacent to the basal corpuscle of the kinocilium. Moreover, small and large granules, membranes of the endoplasmic reticulum and vesicles with a diameter of 400-800 Å located primarily in the region of synapsis, are contained in the cytoplasm of a receptor cell.

Nerve fibers penetrating into the epithelium approach Type I receptor cells and form huge nerve cups of irregular shape in which the bodies of 2-5 receptor cells are submerged almost to the very summit. The axoplasm of these cups is transparent and rich in small mitochondria. Often small dark nerve endings filled with mitochondria and synaptic vesicles lie at the base of the cup. The authors consider that these are axi-axonal nerve endings.

On Type II receptor cells nerve fibers form bud-shaped nerve endings. Sometimes they are deeply immersed in the cytoplasm of receptor cells, almost reaching the nuclei. These endings are light in color and contain small mitochondria in synaptic vesicles. But at the same time there are dark nerve endings containing a large number of synaptic vesicles on these cells.

The authors arrive at the conclusion that the appearance of such gigantic scyphoid synapsis is a new evolutionary adaptation in birds and is connected with their flight and free movement in the gravitational field. /22

Even the cytochemical characteristics of these cells changes (Vinnikov, Gazenko, Titova, Bronstein et al., 1965). While in the cylindrical receptor cells of the vestibular auditory portions there

is preserved a more or less equal distribution of RNA in their cytoplasm, which is more richly present in the auditory receptor cells, for pitcher-shaped vestibular receptor cells, the presence of bright granular accumulations of RNA both below and above the nucleus is characteristic. The distribution of protein and carboxyl groups of protein molecules corresponds with the distribution of nucleic acid, but differs by the very high concentration in the pitcher-shaped receptor cells. In this regard, there is a similarity even in the distribution of thiol groups, only they are more richly represented in the receptor cells of the auditory portions. This especially distinguishes the distribution of thiol groups in receptor epithelium of *Papilla acustica basilaris* by the fact that a sharp increase in concentration of the groups in the locations of bud-shaped nerve endings (Titova, 1966) is present there.

For the neurons of both the vestibular as well as of the cochlear ganglia of birds, a high concentration of nucleic acids, protein, and functional groups of protein molecules is characteristic, which is stronger in the cytoplasm than in the nucleus and is significantly increased in the granules of Nissl's substance.

High activity of ACE is noted in the region of synapsis and receptor structures of the inner ear of birds. However, at the same time there is very high cholinesterase activity in them which reacts with butirilthiocholine, especially in the region of the synapsis and in the secretory zone. It is characteristic that this cholinesterase does not react with acetylcholine and is not inhibited by inhibitors for nonspecific cholinesterase. Basically, only specific cholinesterase is present in neurons of the vestibular and cochlear ganglia.

Enzyme activity of all investigated dehydrogenases, with the exception of LDH, is always connected with mitochondria, located both in the cytoplasm of receptor cells as well as in nerve fibers approaching them. The level of their enzyme activity is very high, especially in the region of the scyphoid synapsis.

In the receptor structures of the inner ear of mammals (cat) we find the same relationship as in the vestibular portion in birds, only their pitcher-shaped cells are usually present in a ratio of one (rarely two) per scyphoid nerve ending. The latter is most often found in the more active animals which move by jumping (monkeys, cats) and has only one basal accumulation of granular RNA. Similar to birds, their pitcher-shaped receptor cells are richer in protein, its functional groups and cylindrical vestibular receptor cells. Both types of receptor cells have a bundle of stereocilia and one mobile kinocilium at their summit.

In the vestibular portion of mammalian labyrinth, high enzyme activity of ACE is observed which, as usual, is located in the synapsis and nerve fibers. Also, even high enzyme activity of nonspecific cholinesterase in the crescent-shaped lamina of the ampulla

cristae is characteristic. The unusually high enzyme activity of ACE is noted also in the neurons of the vestibular ganglion. In Corti's organ, ACE is present along the path of the surface and internal spiral plexi in regions of the synapsis of the nerve endings with the bases of the surface and internal hair cells, whereby, as was already noted, the ACE activity on the upper helixes of the cochlea is only manifested in the regions of the internal plexi, and on the lower helixes, both in the region of the internal and of the external plexi. But there is a principal difference in the distribution of ACE in the cochlea of mammals which consists of the fact that in addition to the usual location of ACE in regions of the synapsis of nerve endings, a very high enzyme activity is manifested in the hairs of the internal hair cells along the entire spiral of the Corti's organ and in the hairs of external hair cells at the level of the lower helixes of the cochlea. ACE activity is observed also in the cytoplasm and in the nucleolus of hair cells of Corti's organ. In neurons of the cochlear ganglion this activity is very moderate.

SDH activity in the receptor structures of the inner ear of mammals is very great. It is located in the mitochondria of all receptor cell bodies and in the nerve fibers approaching them, and is especially intensively manifested in scyphoid nerve endings. The activity of ADH, MDH and LDH is also manifested in the mitochondria, but as in other vertebrates, its activity is much higher in the nerve fibers and in the synapsis than in the body of a receptor cell. As was noted earlier the activity of LDH also has extra mitochondrial location.

The factual material presented above on the chemism of the receptor formation of the inner ear for receptors of various classes of vertebrates revealed a number of clear evolutionary transformations, reflecting not only the complication of their general organization, but also the ecology of animals in regard to their position in the gravitational field. It was shown (Vinnikov, Govardovskiy and Osipova, 1965a), that in the utricle of bony fishes (pike) there are only Type II hair cells, crowned by a bundle of stereocilia, supporting a gigantic otolith and one strictly oriented mobile kinocilia. Type II cells, as was shown by Wersäll (1956) and others (cf. Types of Hair Cells, p. 16), are distinguished by the fact that some bud-shaped light afferents, dark efferents of nerve endings approached their basal end, which are covered with myelin almost to the synaptic membrane.

With the movement of vertebrates onto the dry land with respect to reptiles (Vinnikov, and Titova, 1962) and mammals (Engström, 1960; Wersäll, 1961; Vinnikov, Gazenko, Titova and Bronstein, 1963; Vinnikov, Gazenko, Titova, Bronstein et al., 1965), in addition to the cylindrical receptor cells there appears a modification of hair receptor cells, i.e. the so-called pitcher-shaped receptor cells or Type I cells which, according to our data, attain particular development in birds leading an aerial life. Type I cells are also found

with a bundle of stereocilia supporting an otolith membrane, with small otoliths and otoconia, and one equally oriented mobile kinocilium. But as opposed to Type II cells, Type I cells are immersed in special scyphoid nerve endings, due to which all of their surface plasmatic membranes, with the exception of the summit crowned with hairs, are turned into a synaptic surface of great extent. As a rule, the dark nerve fibers terminate on the external surface of a nerve cup, evidently forming an axo-axonal nerve synapsis to which, at the present time, an inhibitory function is ascribed (Gray, 1962). However, while in the guinea pig there is located one such Type I cell per each such nerve, and in the mouse, cat and monkey there are two of each, in birds the number of Type I receptor cells per one nerve cup may reach 5-7. In addition, each cell of those which are immersed in the general cup can have its own slope and its own spatial orientation, distinguishing it from the neighboring cell. The appearance of several receptor cells located in one nerve cup is accomplished by a sharp complication of their synaptic contacts with the nerve endings. The indicated evolutionary transformations in the structure of the epithelium of the utricular macula evidently reflects the function of the utricle not only as the organ of gravitation, but also as the navigational instrument in birds (Screiber, Qualtierotti and Mainardi, 1962). /24

Analogous evolutionary transformations, as we have seen, also take place in the evolution of Corti's organ in birds and in mammals.

THE FUNCTION OF RECEPTOR FORMATIONS OF THE VESTIBULE

By means of intravital observations it was established that the deflection of the cupula in the ampullas takes place with head movement or with the action of angular acceleration, and is elicited by corresponding motion of the endolymph. However, only motion of the endolymph in a definite direction along the semicircular canal leads to stimulation of the receptor cells of the crista and to nerve excitation, which is accompanied by reflex response of that portion of the skeletal musculature which corrects the position of the body muscles and motion of ocular muscles (Magnus, 1962). Excitation of receptor cells of the cristae of the semicircular canal is accompanied by the appearance of biocurrents. The action currents arise in the nerve fibers during depolarization of the synaptic membrane connected with them. In the crista of the ampulla of the horizontal canal, centropetal stimulation, i.e. deflection of the cupula in the direction toward the utricle, is accompanied by an elevation of action current frequency on the part of the nerve fibers. Deflection of the cupula in the opposite direction, i.e., in the direction toward the cavity of the semicircular canal, is accompanied by a lowering of the action current frequency. In the ampullar cristae the reverse relationships are observed. As was already noted, in the crista of the horizontal canal the kinocilia of receptor cells are oriented in the direction toward the utricle, while in the cristae of the vertical canals, they are oriented in the opposite direction, i.e., toward the open space of the semicircular canal

(Löwenstein and Wersäll, 1959). These facts show that undoubtedly there is a connection between the polar distribution of the kinocilium on the surface of the receptor cell and its function. Deflection of the cupula toward the kinocilium excites the cell and leads to its depolarization in the region of synapsis. Deflection of the cupula towards the stereocilia leads to hyperpolarization and to the inhibition of the cell. However, in addition, the mechanical shifts which are at the basis of the appearance of potentials or their inhibition, i.e., the specific role of the surface membranes of the stereocilia and the kinocilia, remains unclear.

A detailed investigation of the distribution and orientation of stereocilia and kinocilia on the surface of the receptor cell (Flock, 1964) showed that the stereocilia are usually located in 7-9 regular rows opposite the kinocilium. The distance between the rows is greater than the distance between the neighboring stereocilia of the same row. The kinocilium is located somewhat back along the edge of the first two rows of stereocilia, which form a notch for it in the form of an open angle of 115° . Inside the bundle the length of a stereocilium gradually increases in the direction toward the kinocilium. The kinocilium is longer than the stereocilia.

The polar orientation of the kinocilium in receptor cells of the utricular macula was recently studied in detail on selachians and bony fishes (Löwenstein et al., 1964; Flock, 1964). It is possible to consider that, just as in the crista of the semicircular canal, polarization of the kinocilium in the utricular macula reflects the direction of stimulation for each receptor cell separately. /25

Receptor cells are oriented in the utricular macula in radial rows. Rows of receptor cells in the central portion of the macula diverge laterally in semicircles in the direction towards its anterior and posterior borders in such a way that the kinocilia originally oriented toward the lateral and partially posterior surface gradually change in direction toward the anterior surface. In the border zone of the macula, the kinocilia drawn along its anterior lateral and posterior edge are oriented in the opposite direction, toward the kinocilium of receptor cells of the central portion. Thus the fundamental direction of orientation of the receptor cell kinocilia in the utricular macula corresponds to a laterally oriented line. The latter forms a 30° angle with the utricular nerve, which approaches from below. By analogy with the cristae of the semicircular canals and the lateral line organs (Flock and Wersäll, 1962b), it is possible to assume that a receptor cell of the utricular macula is excited if stimulation is directed toward the stereocilia and, in addition, a depolarization of synapses takes place, accompanied by an elevation of the impulse frequency in nerve fibers. If the stimulation is in the opposite direction, i.e. toward the kinocilia, hyperpolarization of synapses and inhibition, i.e. a lowering in the impulsation frequency of the nerve fibers, is observed.

Löwenstein and Roberts (1949) studied the influence of a change in body position to the frequency of action currents conducted from isolated nerve fibers of the utricle of a skate. They discovered a large correspondence between elevation and inhibition of action currents and any inclinations of the animal's body. It proved to be the case that in the skate utricle there are two types of nerve endings: some respond with an increase in action current to the position of the body with the side and nose upward; others to the body position with side and nose downward. The first are located in the anterior lateral portions of the utricular macula; the second in the posterior lateral portion which corresponds on Flock's scheme (Flock, 1964) of location of polarized receptor cells to the peripheral portion of the utricular macula of bony fishes. Unfortunately, to date the experiments of Löwenstein and Roberts (1949) have not been successfully repeated on representatives of other vertebrates, due to the difficulty in attaining access to the organ. In this regard, meanwhile, it is necessary to rely on indirect data obtained with the conduction of biopotentials from the vestibular nuclei of the brain stem.

Adrian (1949) succeeded in showing that gravitational changes corresponding to lateral and anterior-posterior inclinations of a cat's head are accompanied by an elevation in impulsation changing with "sideways-downward" inclinations of the corresponding side. Experiments performed on vestibulae of bony fishes and on several representatives of mammals gave analogous results (Gernandt, 1949, 1959; Ruppert et al., 1962; Gazenko et al., 1962). Polarization of receptor cells undoubtedly must reflect the functional interaction of various portions of the utricular macula. In Flock's opinion (Flock, 1964), the inclination of the utricular macula around a normal axis to the right, i.e. "sideways-downward", may serve to illustrate this interaction. In the right utricle such an inclination leads to lateral slippage of the otolith membrane and the development of "cutting forces", thereby stimulating cells of the central portion of the utricular macula. At the same time, these forces inhibit receptor cells of the peripheral zone of the utricle. In these zones, as was already mentioned, the kinocilia are oriented in the opposite direction. In the left utricle an inclination in the same direction must elicit slippage of the otolith toward the center, and the "cutting forces" which appear stimulate the receptor cells, which are a mirror image of those in the right utricle. Hence it is possible to draw the conclusion that the characteristics of the peripheral zones of both utricular maculae are opposite each other, but similar to the characteristics of the central portion of the opposite macula. Likewise, the characteristics of the central portions of both utricular maculae are opposite to each other, but similar to the characteristics of the peripheral zones of the utricular macula on the opposite side. /26

A corresponding effect in motion "before" and "after" inclination around the horizontal axis connects both utricular maculae. In addition, inclination in the "nose downward" direction is accompa-

nied in each utricular macula by excitation of the receptor cells in the anterior central portion and in the posterior peripheral zone and by inhibition of the posterior central portion and anterior peripheral zone. Consequently, the anterior and posterior portions of each utricular macula possess opposite characteristics but they will both signal a change in the position of the body due to their similar structural characteristics. Thus, in the organ of gravitation, direction of reception completely corresponds to efferent inclination which consists of antagonists, capable of both homolateral and contralateral interaction. Hence, the very contradictory results obtained by many investigators in studying the functions of the utricle with the aid of action by mechanical centrifugal forces, including the partial or complete removal of the labyrinth, are made understandable. Complete unilateral removal of the labyrinth leads, as is known, to an asymmetry of tone which is expressed in an overbalance of antigravitational muscles on the intact side.

The investigations of Magnus (1924; 1962) and Versteegh (1927) showed that adjusting labyrinthal reflexes begin in the utricular macula. The observations of Tait and McNally (1934) on a frog from which they removed all the receptor organs of the labyrinth, confirmed the homolateral overbalance of antigravitational muscles under the influence of the intact utricle. Contradictory results obtained by Löwenstein and Roberts (1950) on one side and by Adrian (1949) on the other may possibly be explained by the characteristics of polar organization of receptor cells of the utricular macula.

Tait and McNally (1934) and Mygind (1952), as a result of the investigation on a frog with one intact utricle, came to the conclusion that the utricular macula possesses two opposite functions, one of which is primary, the other subordinate. Mygind explained their intersecting connections from the opposite side.

The regularities of polar asymmetry in the distribution of the kinocilia in the utricular macula and their functional parallelism, in comparison with the more distinct data on the lateral line and ampullar cristae of the semicircular canals, permit us to describe the direction of stimulation. But the nature of this stimulation remains unclear.

Until recently the process taking place in the receptor cells of the utricular macula themselves during their stimulation remained completely unknown. Recent investigations conducted in our laboratory (Vinnikov, 1964; Vinnikov, Titova, Gazenko, and Bronstein, 1963; Vinnikov, Gazenko, Titova, Osipova et al., 1963) showed that during such stimulation of the utricular receptor cells there is first a shift on the part of the substructural cytochemical organization of the nucleus in cytoplasm, which will be analyzed in detail with a description of our own data. This is expressed by a shift in the direction of the nucleolus toward the nuclear membrane and the transfer of ribosomes contained in it through the pores of the nuclear membrane into the cytoplasm, where they induce a limited

endoplasmatic net, which in light microscopy appears as an "escaped" nucleolus. These processes take place especially demonstratively in the utricular macula of the guinea pig, in monkeys and in birds. Such a movement of nucleolic RNA into the cytoplasm, possibly bearing informational RNA, evidently represents a rhythmic process connected with general protein synthesis, which may be disturbed under experimental action and loses its synchronicity in specific receptor cells. A cytophotometric investigation showed that after the reconstruction of cytoplasmic RNA, a process of elevation of protein synthesis begins which reaches a certain maximum, then falls almost to the normal level, and again is elevated, forming a multi-peaked curve with the highest point two days after a single experimental action. Repeated experimental action has an unfavorable influence on the maximum points of protein synthesis, eliciting disturbance or even cessation of it. Repeated experimental action at those times when the construction of RNA takes place elicits the greatest effect of strengthening protein synthesis, but protein synthesis still did not begin (8 hours, 3 and 6 days after single action). The critical stage is 5 days after a single action. Repeated action at this stage elicits a sharp lowering in protein synthesis accompanied by vacuolation of the cytoplasm and nucleus.

Thus, in the receptor cells of the utricle of a guinea pig, monkey and pigeon, with their stimulation by radial acceleration, a regular cycle of cytochemical shifts is observed, connected with the protein synthetic function of nucleic acid (RNA). The change in the protein cycle of receptor cells corresponds to the change (elevation in the initial and decrease in the final periods of the experimental action) in the activity of a number of enzymes. This relates to the oxidized enzymes located in the mitochondria and to the acetylcholinesterase located in the synapsis (Vinnikov and Titova, 1962; Titova and Vinnikov, 1964). These cytochemical data at the present time have been successfully specified with the aid of the electron microscope. In the region of the synapsis of the receptor cells of the utricle, synaptic vesicles and characteristic rod-shaped presynaptic membranes are revealed. Synaptic vesicles may densely border the presynaptic membrane, and furthermore they may discharge their contents into the intersynaptic fissure. In several cases a movement of synaptic vesicles is observed, as well as its penetration through the synaptic membranes into the nerve endings lying below. On the contrary, sometimes one observes the penetration of synaptic vesicles from the dark nerve endings through the synaptic membrane into the cytoplasm of a receptor cell. With experimental actions a sharp increase in the number and size of synaptic vesicles and their motion to the synaptic membranes was observed.

Changes in the mitochondria of the synaptic regions were very demonstrative in the electron microscope. Under conditions of relative rest, presynaptic mitochondria, more or less equal in size, were distinguished slightly by the concave form, density of distribution of cristae and dark matrix. Several of these mitochondria /28 were found on the surface of the presynaptic membrane. Postsynaptic

mitochondria were differentiated from them both by size and shape. Some of them were large and round, others were smaller and of rod-like form. The action of radial acceleration on the animal leads to various changes in the position of mitochondria and their structure. This especially relates to the Type I receptor cells surrounded by scyphoid nerve endings in the utricle of mice. Practically all the mitochondria in the presynaptic region are slightly swollen, straightened, and elongated; approaching the presynaptic membrane, they accumulate on its surface. At the places of mitochondria accumulation the number of membranes correspondingly increases by 4 times. The postsynaptic mitochondria of scyphoid nerve endings can be divided into two types. The first type includes mitochondria of small size with thickly located crests and a high electron density of the matrix. The second type are large round mitochondria, occupying almost the entire space of the cup. They are distinguished by thin severed tube-like crests and transparent matrix. Possibly, the second type of mitochondria are the result of the swelling of the first type of mitochondria. As is known, swelling of the mitochondria reflects their participation in the process of glycolysis (Neyfakh, 1965). The effect of mitochondria application to the presynaptic membrane is observed even on the part of dark (efferent) nerve endings. These electron microscopic and cytochemical data allowed us to draw the conclusion that in the process of excitation conduction, besides the specific cholinergic mechanism, a nonspecific oxidizing cycle also participates (Vinnikov, Gazenko et al., 1966).

We have permitted ourselves to deal briefly here with the basic investigations of our laboratory, dedicated to deciphering the mechanisms of excitation of the utricular macula. Several of these will be dealt with in greater detail in the section devoted to an investigation of the functional changes in receptor structures of the inner ear of vertebrates.

Structure of the Cochlea in Birds

In the higher representatives of the Sauropsida (crocodiles and birds) the membranous labyrinth in the region of the sacculus has a long sac-like blind outgrowth, i.e., the cochlear canal, homologous to the cochlea of mammals. Along the length of the cochlear canal on a basilar membrane attached to the skeletal frame is located an elongated acoustic papilla (*Papilla acustica basilaris*) containing receptor hair cells, specialized for the perception of auditory isolations. At the blind end of the cochlear canal in crocodiles, birds and monotreme mammals, the lagena is observed in its own sensory spot (*Papilla acustica lagena*), preserving its otolith apparatus. In birds a special covering lamina is developed on the basal papilla, increasing its resemblance to Corti's organ in mammals.

The first investigation of the avian cochlea was conducted in the 17th century by two investigators: Casserius (1600) and Per-rault (1680). In the 19th century, Treviranus (1825) compared it with the mammalian cochlea. The position of the cochlear canal in

the temporal bone of birds was studied by Breschet (1833), and its anatomical structure was first investigated in detail by Hasse (1867). The fine structure of the cochlear canal was studied in detail by Hannover (1842), Leidig (1857), Deiters (1860) and others. These investigations involved extensive comparisons between the avian cochlea and the mammalian cochlea which proved to be correct even in present investigations. /29

If we consider the cross section of the avian cochlear canal, then it is possible to see that on the upper surface it is bounded by the so-called vascular tegment (*Tegmentum vasculosum*), which contains both the vascular stria and Reissner's membrane in mammals. The vascular tegment divides the vestibular ladder, the presence of which is denied by Schwartzkopff (1963). Under the cochlear canal or to its side is located the tympanic ladder. It is located under the basilar membrane, which in turn is stretched by means of a special cartilaginous frame. The vestibular ladder adjoins the cochlear window (*Fenestra cochlea*), which is covered from the side of the tympanic cavity of the inner ear by a mucous membrane, i.e., the secondary tympanic membrane (*Membrana timpani secundaria*). In the cavity of the middle ear, or the tympanic cavity, there is a columella, which, by means of its own expanded base (*planta*), is inserted into the oval window of the labyrinth, leading to the vestibular ladder. When this columella connected to the tympanic membrane transmits air vibrations to the perilymph of the vestibular ladder, oscillatory motions in the endolymph of the cochlear canal arise. This motion encompasses the cavity of the cochlear canal with the basilar acoustic papilla, i.e., with the Corti's organ of birds. The motion of the papilla located on the basilar membrane is transmitted to the perilymph by the tympanic membrane. The avian helicotrema is, in all probability, functionally identical to the mammalian cochlea, intersecting the cartilaginous knee above the basilar membrane in the *tegmentum vasculosum* (Gray, 1907-1908, as cited by Werner, 1960; Schwartzkopff and Winter, 1960).

The avian cochlea is quite well developed, although it does not form complex spiral turns as in mammals and makes two small bends (Gadow, 1891; Stresemann, 1934) originally around the transverse axis and spirally circling along the long axis. Anatomically the cochlea is subdivided into a superior larger part, the *Pars basilaris cochlea*, and a narrowed pocket end, the *Lagena cochlea*. In the region of the *Pars basilaris cochlea*, the cochlear canal has a more or less equal width, then is reconnected in a neck-like way and, in the region of the *Lagena cochlea*, it is expanded like an ampulla. The latter is completely surrounded by a thin osseous capsule, the former (*Pars basilaris cochlea*) in the osseous labyrinth corresponds to ligaments (the so-called cartilaginous knees): the posterior triangular and the anterior rectangular or conducting, in which there are openings for the passage of the terminals of the auditory nerves into the labyrinth and where part of the ganglion cochlearis is located (Deiters, 1860; Ramon and Cajal, 1909).

The cavity of the Scala tympani of the avian cochlea has a very complex structure (Retzius, 1884; Krause, 1906, 1922; Satoh, 1917; Werner, 1938a; Schwartzkopff and Winter, 1960). Generally the following parts are differentiated in it: the Recessus scala tympani (bordered by the round window); the Scala tympani (under the basal portion of the basilar membrane) and the Cavum scala tympani (under the apical end of the basilar membrane); the Ductus scala tympani (under the Cavum scala tympani between the ligament and the bone leading to the helicotrema); the Ductus brevis and the basal union of the membranes of the Scala, similar to the apical Ductus scala tympani (Schwartzkopff and Winter, 1960).

The vestibular wall of the cochlear canal according to Held (1926) consists of an interfibrous tissue, providing thin traces to the bone and covered with a flat endothelium on the side of the vestibular cavity. The blood vessels pass in the connective tissue traces, penetrating into the epithelium of the vascular tegment. Epithelium lines the internal surface of the vestibular wall and turns into the cavity of the canal. In addition, the connective tissue forms a series of complex folds, penetrated by blood capillaries, due to which the epithelial surface of the vascular tegment appears to be very unequal and significantly increased in size. Up to now, due to these complicated folds it was not possible to establish whether the epithelium of the vascular tegment is one-layered or many-layered. A cross section of the folds shows the epithelium to be one-layered and not connected with blood vessels. It consists of two types of cells: (1) light cylindrical cells with a polygonal cross section, passing through the entire epithelium up to the surface; (2) cells having a flask-like shape containing granules and grains of pigment in their expanded basal portion. Their homogenous or slightly vacuolized neck is located at the surface of the epithelium. Held assumes that the latter elements are secretory in nature. In the cytoplasm pigment inclusions are also revealed. According to Stresemann's data (Stresemann, 1934) they fulfill a secretory function and as dialyzers participate in the production of endolymph. Werner (1940) mentions that their bodies barely reach the base of the epithelium. According to Held (1926), these cells have a round or star-shaped form and anastomosing processes. Their nuclei are located in the neck near the surface. Held also remarked that they contain a diplosome (basal corpuscle) with a flagellum. /30

In the region of the anterior wall of the canal, elements of the vascular tegment are leveled out and form one layer of transparent cylindrical epithelial cells. To these elements is attached the tectorial membrane into which they send short processes from their apical surface. Satoh (1917) calls these cells "homogeneous"; Deiters (1860) designated them as "cylindrical bodies"; Hasse (1867) used the term "tooth cells". They correspond to the epithelial cells of the Labium vestibular in the mammalian cochlea. A similar group of cells located on the posterior cartilage does not have any relation to the tectorial membrane but has the same homogeneous cytoplasm. Behind the posterior and anterior homogeneous cells and on

the anterior and posterior ends of the basilar membranes are located light hyaline cells; the anterior ones are higher, the posterior ones are lower cylindrical cells. In the opinion of Held (1926), the first correspond to the cell of the Sulcus spiralis of mammals, and the group of low hyaline cells correspond to Hensen's cells.

The Papilla acustica basilaris of birds corresponds to Corti's organs in mammals; i.e. it is homologous. The basilar membrane, hair cells, auxiliary supporting cells, nerve fibers and the tectorial membrane are basic parts of the avian organ of hearing, just as the mammalian Corti's organ. However, despite the presence of a similarity in the distribution of homologous parts there are substantial differences between the avian Papilla acustica basilaris and the mammalian Corti's organ. The basilar membrane of birds is proportionally broader and significantly shorter than that of mammals. It stretches above the surface of the conducting rectangular cartilage and up to the triangular cartilage. The sensory epithelium located on it is significantly broader than that of mammals, and its cross section in the pigeon contains 15-25, in the rooster 27-32, and in the parrot 28-33 sensory cells (Kolmer, 1927); the tunnel and other intraepithelial spaces in birds are completely absent.

The Papilla acustica basilaris is a broad depressed epithelial formation, one-third of which is located on the cartilaginous lamina with its neural knee. The remaining 2/3 are located on the basilar membrane. The basilar membrane itself is stretched between the lip-like sharpened edges of the cartilaginous lamina. In addition, the fibrous matter of the cartilage directly turns into the fibers of the basilar membrane (Held, 1926). Held distinguishes two zones in the avian basilar membrane: the anterior and the posterior. On the anterior zone is located the larger half of the basilar acoustic papilla, and on the posterior a unique group of low light cells, which were discussed above. In accordance with the fact that the avian cochlear canal is pear-shaped, the basilar membrane to its summit is gradually expanded, in the hen from 66 to 400 μ (Held, 1926), but it narrows again toward the end. /31

In the basilar membrane an upper transparent layer is distinguished. It is thin and appears as a basal membrane on which the acoustic epithelium of the papilla is located. Then two fibrous layers are found: the upper having thin fibers and the lower having thicker fibers. The fibers are located in a homogeneous basal substance in which individual nuclei of some connective tissue cells are occasionally found. From the side of the tympanic ladder the membrane is covered with endothelium. The fibers of the basilar membrane consist of collagen, and in the posterior portion even elastic fibers are observed. The number of fibers in the basilar membrane of the chicken is 1200; i.e., 1/20-th of the fibers of the basilar membrane of man. According to the data of Werner (1940), Dement'yev (1940) and Freye (1952-1953), the avian basilar membrane contains irregularly oriented threads in its deepest layers. In the upper layers it consists of extremely elongated threads, located

parallel to the surface, which from 2/3 to 3/4 of the membrane remain freely stretched on the cartilaginous frame. The anterior and posterior zones in cross section have a weakly lenticular shape. On the anterior zone rests the greater portion of the Papilla acustica basilaris, and on the posterior zone is a group of low hyaline cells. The basilar membrane in both zones consists of: a very thin upper layer separating the basilar membrane from the receptor layer; two fibrous layers, an upper one consisting of thin fibers and a lower with thick fibers which are subdivided into thin homogeneous layers; and finally the connective tissue endothelial cells, forming a layer corresponding to the tympanic lining layer. The fibers of the basilar membrane are surrounded with a light homogeneous intermediary substance in which are located the extended nuclei of connective tissue cells, the processes of which, according to Hasse (1867), form these fibers. A difference in the staining of fibers of the anterior and posterior zones of the basilar membrane is noted. Held (1926) notes that in the anterior zone they are stained like collagen fibers, and in the posterior zone like elastic fibers.

Unfortunately there are not very many studies dedicated to the cochlea of birds, basically dealing either with the anatomical structure or with its physiology (Werner, 1940; Freye, 1952-1953; Schwartzkopff and Winter, 1960; Schwartzkopff, 1960; Wewer et al., 1959, and others). A more precise and detailed description of the Papilla acustica basilaris in birds is found in the investigation of Held (1926). But it goes without saying that this description, however precise and detailed, is dated by more than 40 years and cannot completely satisfy current demands. However, it is necessary to note that the many discoveries Held made with the aid of light microscopy methods were rediscovered recently with the aid of the electron microscope (for example, the presence of kinocilia in receptor cells of the Papilla acustica basilaris of the pigeon).

The basilar acoustic papilla (Papilla acustica basilaris) in birds consists of low receptor cells, alternating with high supporting cells. The receptor cells on their apical surface, have the so-called hair lamina, i.e. cuticle (Held, 1926), from which sensory hairs proceed. Supporting cells are distinguished by the presence of tonofibrils extending along their entire length. The cells directly adjoin the basal membrane by their narrow bases. Between the bases of supporting cells are located nerve fibers, i.e., neuron dendrites of the eighth auditory ganglion. /32

Near the summit the supporting cells are dispersed and the bodies of receptor cells are located between them, on the basis of which the already mentioned nerve fibers form synapses.

The nuclei of supporting cells are located closer to the base in some of the cells, and in others on the middle and higher levels of the epithelial layer, depending on the type of bird.

In the papilla acustica basilaris of the pigeon Held (1926)

described a certain type of tunnel which is formed at the point of entrance of dendrite fibers of the auditory neurons due to the rimosose departure of supporting cells from one another.

Receptor elements are cylindrical epithelial cells. Their height somewhat predominates in the anterior and posterior portions of the papilla. They are distinguished by a round base and smooth apical surface, provided with a bundle of auditory fibers. The cytoplasm on fixed preparations is granulated; the nucleus is oval and located nearer to the base. The hairs are usually glued together. The number of hairs may vary and reaches 100. Held (1926) first described the basal corpuscle (diplosome with a flagellum located behind the hairs in the pigeon). Recently this was confirmed with the aid of the electron microscope (Vinnikov, Osipova, Titova and Govardovskiy, 1965).

Receptor cells of the avian Corti's organ have a very uniform structure. They belong to Type II hair cells, and at their apical end have a bundle of stereocilia and one mobile kinocilium oriented interpolarly, having a characteristic fibrillar structure consisting of 9 pairs of peripheral and two central fibrils. Their stereocilia are attached to the tectorial membrane by their summit with the aid of a special glue. At the base of the receptor cell lies a round body of light and dark nerve endings, performing a synaptic structure on them. The dark endings evidently are efferents (Boord, 1961).

The branching of the auditory nerve is especially rich at the beginning of the Papilla acustica basilaris. Characteristic of the avian cochlea is the fact that the auditory nerve innervates not only the hair auditory cells, but also the posterior hyaline cells, while in the mammalian cochlea, the auditory nerve endings do not extend beyond the external hair cells (Held, 1926).

Thus, in birds there is not yet a subdivision of receptor cells into external and internal hair cells, which causes us to be careful in making a complete homology of the physiology of the hearing of birds and mammals.

The tectorial membrane at the Papilla acustica basilaris of birds is similar in structure to that of mammals. It starts from homogeneous high cylindrical cells which, in the cochlea of mammals, correspond to the cells of the Limbus spiralis. The region corresponding to the internal spiral incisure of mammals is severely reduced (Werner, 1940) or completely absent in birds, and the tectorial membrane stretches like a blanket over the surface of the hair cells (Retzius, 1884; Pumphrey, 1961). The early authors (Sato, 1917; Breuer, 1907) considered that the sensory hairs of receptor cells either were directly connected or turned directly into fibers of the tectorial membrane. But Held (1926) refutes these data and shows that the tectorial membrane of birds forms teeth on its lower surface, by which it is attached to the free surface of the support-

ing cells, forming a clear helmet-shaped space above the hair bundles (Meyer, 1870; Held, 1926). However, as our electron microscope data show, the summits of the stereocilia are attached to the internal surface of the tectorial membrane by means of a special cement (Vinnikov, Osipova, Titova and Govardovskiy, 1965).

In relation to the thin structure of the tectorial membrane in birds it is only known that it consists of thin and thicker interwoven fibers, glued with an interstitial basic substance. On its concave exterior surface it is covered with a certain thin cuticle (Held, 1926). The lateral borders of it are attached to the anterior transparent cells, the processes of which enter for a certain distance into the tectorial membrane. Evidently these cells regulate the tropicity of the tectorial membrane. As the investigation of Belanger (1953) showed, mucopolysaccharides are contained in the enzyme. In embryogenesis the tectorial membrane is differentiated due to the activity of these cells.

There are no electron microscope investigations on the thin structure of the *Papilla acustica basilaris* of birds, with the exception of those made in our laboratory (Vinnikov, Osipova, Titova, Govardovskiy, 1965).

The literary data given above show the extreme scarcity of information on the thin structure of the hearing organ of birds, the *Papilla acustica basilaris*. However, for investigations into the nature of acoustic perception, these data are extremely significant, insofar as the hearing organ of birds is not a predecessor of the hearing organ of mammals, and both of these organs developed independently from one common base. This divergence began, evidently, not later than in the high carboniferous period (250,000,000 years ago) and from that time these two organs developed independently (Pumphrey, 1961).

It is generally accepted at the present time that birds far surpass other animals in the development of the hearing organ, but in no way does it follow from this that this superiority in both classes in the process of evolution was reached by one and the same means. Helmholtz (1868) considered that in both classes this superiority depends partially on the peripheral process and partially on analysis, which in turn is connected with the psychophysiological ability to distinguish the pitch of a sound. The presence of structures, distinguished to some degree by their thin construction, whose operation leads to one result (i.e., frequency perception and analysis of sound) is an invaluable experiment set on the grandiose scale of nature itself, which provides the investigator with material for obtaining an answer to the question of the mechanism of auditory perception (Pumphrey, 1961).

Thus, at the basis of the perceiving structures of the inner ear in vertebrates, despite the very complex nature of its anatomical structure, lies the receptor cell, provided with a bundle of

stereocilia and one polarly located mobile kinocilium, which is absent only in the receptor cells of Corti's organ of mammals. Above the stereocilia and the kinocilium a covering structure is developed in the form of an otolith membrane with otoliths, or a cupula in the vestibular portion of the inner ear, or a tectorial membrane in Corti's organ. Depending on the nature of innervation, Type I cells with scyphoid synapses and Type II cells with bud-like synapses are distinguished. Consequently, in addition to a structural change in one or another receptor formations of the inner ear connected with the change or "segmentation" in its function (utricle: organ of gravitation; saccule: organ of vibration; ampulla of the semicircular canal: organ receiving angular accelerations; cochlea: organ of hearing), in the process of evolution there is observed also a change in the nature of their innervation, i.e. the synaptic structures, which reflects an ability for transmission of cellular information into the central nervous system and the presence of efferent innervation along with the afferent innervation. However, Type I cells indicate a later stage of evolution; they are absent in the receptor structures of the inner ear of the lower vertebrates and are exarticulated first in the receptor structures of the inner ear of reptiles. In this regard, the receptor formations of the inner ear of the lower vertebrates are structurally closer to the receptor formations of their lateral line organs, at the basis of which also lie Type II receptor cells. In the process of further evolution of the receptor formations of the inner ear with the exarticulation of a new receptor structure, i.e., the organ of hearing (Corti's organ), a further evolution of auditory receptor cells takes place on the basis of this Type II receptor cell with bud-shaped nerve endings. The Type I vestibular receptor cell with scyphoid nerve endings, being more differentiated and specialized, evidently loses the ability for further evolution.

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As far as supporting cells are concerned, their structure remains practically unchanged in the receptor organs of the vestibule of the labyrinth but provides a number of new divergent forms of Corti's organ in birds and mammals.

As was indicated above, the Corti's organ of birds differs sharply from that of mammals both by its general construction and by the structure of its receptor elements. On a cross section of the avian Corti's organ, receptor cells form a very thin and distinct layer of short cylindrical cells. The nuclei of all receptor cells are located on one level. From the side of the auxiliary anterior hyaline and homogeneous cells the receptor epithelium is differentiated by its great thickness, chiefly due to supporting cells, but even here receptor cells are somewhat greater in length. The thickness of the receptor epithelium greatly diminishes toward the end posterior hyaline and homogeneous auxiliary cells, again basically due to supporting cells; however, even the receptor cells themselves are of lesser height here. Along the breadth of Corti's organ the receptor cells are located unequally: while in the anterior portion of the receptor epithelium the receptor cells are densely grouped,

so that only the narrow processes of the phalanges of the supporting cells are located between them, in the posterior half of the receptor epithelium they form individual, still more numerous groups, between which are located the broad upper portions of a supporting cell body. As a result, according to the decrease in the number of receptor cells in individual groups, the number of supporting cells located between them increases. A receptor cell has a cylindrical shape and a cuticular lamina is located at its summit, which under a light microscope has the shape of a small collar. As the electron microscope investigations of Vinnikov, Govardovskiy and Osipova (1965b) showed, the cuticular lamina does not occupy the entire apical surface of the cell, and leaves a significant free portion on one side. From the cuticle only immobile stereocilia proceed, while the kinocilium proceeds from the basal corpuscle, located under the portion of the surface of the receptor cell which is free from cuticle. On their distal ends the stereocilia terminate in blunt capitelli, the summits of which are distinguished by their high electron density. The capitelli of stereocilia seem to be inserted in a notch of the inner surface of the tectorial membrane. Between the membrane substance and the capitellum of the stereocilia there is a light homogeneous lining, which is evidently a cement attaching each stereocilia to the tectorial membrane. Thus the stereocilia are reliably attached by their distal end to the inner surface of the tectorial membrane. /35

As was already noted in the description of the results of electron microscope investigations into embryological material, the kinocilium of all receptor cells of Corti's organ has the same orientation, located back of the bundle of stereocilia, i.e., from the side of the posterior hyaline cells. However, in contrast to receptor cells of the vestibular portion of the labyrinth of other vertebrates, in the receptor cells of the avian Corti's organ a free portion of the surface remains even from the opposite side of the cuticle, which still has not been explained from the point of view of cell polarization or the characteristics of its morphological structure. The cytoplasm of receptor cells differs by its darker coloration, and contains a large number of mitochondria having the shape of short or branching rods, elements of Golgi's apparatus, ribosomes and synaptic vesicles in the region of the synapsis. Elements of a rough endoplasmic reticulum are poorly pronounced. Mitochondria forms two accumulations: under the cuticle and under the nucleus. The nucleus has a regular round shape, small-grained chromatin and a nucleolus which is dark in electron density. The nucleus is covered with a double nuclear membrane.

The supporting cells are characterized by a significantly lighter cytoplasm. They form a multiple layer; the basis of the cells rests on the basal membrane. Expanding in a bulbar manner, in their central portion they form a depression where the bodies of receptor cells are located. Narrowing again, they pass between the lateral sides of the receptor cell and are pushed forward onto the surface of the organ by means of the small intumescences of the cap-

itellum mentioned earlier, the so-called phalanges. The phalanges border the hair cells on all sides. They bear on their surface numerous microvilli, diverging in the form of a rosette. In the cytoplasm of supporting cells there are occasional mitochondria, ribosomes and elements of Golgi's apparatus.

The tectorial membrane located above the summits of receptor cells has a coarse-haired structure under a light microscope. The thickest part of the receptor epithelium lies on the cartilaginous frame above the cochlear ganglion located beside the cartilage, from the neurons of which proceed a large number of nerve fibers passing through the cartilage and connective tissue and between the bodies of supporting cells, forming small bud-shaped nerve endings at the base of the receptor cells. The nerve endings and plasmatic membranes in the region of the receptor cell bases, as may be seen under an electron microscope, forms the synapsis: Some of them are large and light and are distinguished by their light neuroplasm and contain large mitochondria. Evidently these are afferent. Others are small, dark, filled with synaptic vesicles and evidently are efferent. Only the fine constricted portion of the receptor epithelium, containing more densely distributed receptor cells, and also hyaline and homogeneous cells are located at the loosely drawn basilar membrane.

The roof of the cochlear canal is formed by the folds of the Tegmentum vasculosum hanging in its open space. In a fixed cochlea these folds reached the very surface of the tectorial membrane. By staining with ferric hemotoxaline, it is clearly seen that between /36 the cells composing it there are dark and light cells which were covered in detail in the literary survey. The dark cells, as a rule, lie along the periphery of the fold. In its center there is usually a vessel surrounded by a layer of connective tissue. The cells of the Tegmentum vasculosum are differentiated by a thin large body with a round or polygonal shape, and round nuclei in which the achromatin reticulum is clearly seen; well-pronounced grains of chromatin of various sizes are more or less equally distributed in its knots. Sometimes blood cells are visible in the central vessel.

Neurons of the cochlear ganglion are distinguished by a rather small and often irregularly shaped cell body, and by the large round or more oval nucleus. In the peripheral portions of the cytoplasm, Nissl's substance is well pronounced. In the nucleus small grains of chromatin are equally distributed from the light karyoplasm. The nucleolus, located most often in the center of the nucleus, is distinguished from the chromatin by its regular round form and brighter coloration. Upon reaction to nucleic acids one notes a sharp difference between receptor cells, rich in nucleic acids, and the supporting cells, which are very poor in them. According to Brachet, with a reaction, despite a sufficiently significant concentration of RNA diffusely distributed in the cytoplasm, in the subnuclear region of the receptor cell is located a large accumulation of granular RNA, which up to the present time we have observed only in pitcher-

shaped receptor cells of the vestibular apparatus. The nuclei of receptor cells of Corti's organ in birds sometimes have a slightly stained karyoplasm. At the same time, in several nuclei it is completely colorless. The chromatin in the nucleus is distributed equally in the form of average sized grains. The nucleolus, generally lying in the center of the nucleus, has centrally distributed RNA, surrounded on the periphery by DNA. The cytoplasm of supporting cells is distinguished by an insignificant concentration of RNA, which creates a large contrast between supporting and receptor cells. The nuclei of supporting cells appear to be significantly lighter than the nuclei of receptor cells due to the absence of coloration in the karyoplasm and a weaker concentration of DNA in the chromatin. The small nucleolus is distinguished by a higher concentration of RNA, which creates a large contrast between supporting and receptor cells. The nuclei of supporting cells appear to be significantly lighter than the nuclei of receptor cells due to the absence of coloration in the karyoplasm and a weaker concentration of DNA in the chromatin. The small nucleolus is distinguished by a higher concentration of nucleic acids. The cuticle of receptor cells and the tectorial membrane lacks nucleic acids, but are quite intensively colored by nonspecific pyronin.

The cytoplasm of cells of the Tegmentum vasculosum gives a very weak reaction in nucleic acids which evidently attests to the very low concentration of RNA in it. On the other hand, the nuclei of these cells with a very light karyoplasm reveal a significant concentration of DNA in chromatin grains and in the nucleolus. Due to the small concentration of RNA on preparations treated with gallocyenin, we did not succeed in detecting a difference in the RNA content in the cytoplasm of dark and light cells, i.e., in general they appear to be equally stained. Some difference between them can be discerned on preparations treated according to Brachet's method, evidently due to nonspecific tinting of the cytoplasm of dark cells by pyrenin. However, this difference is very insignificant.

In neurons of the cochlear ganglion a very high concentration of RNA in the entire cytoplasm is observed, increasing especially in granules of Nissl's substance. The karyoplasm of nuclei of the neurons lacks nucleic acid, while in the chromatin veins a very high concentration of DNA is observed. Likewise, the nucleoli of these neurons are rich in nucleic acids. However, among the neurons of the cochlear ganglion, the level of concentration of nucleic acids is not always the same; and even within the limits of one section of an intact animal it is possible to see neurons which are richer in RNA and those which are poorer in it. RNA is absent in the processes of the nerve cells. /37

There is a difference between the cytochemical characteristics of receptor and supporting cells on preparations treated for total protein according to Danielli's method. Receptor cells here form a dark row, which stands out sharply against the light background of supporting cells. In the cytoplasm of receptor cells an average or

somewhat higher than average concentration of total protein is observed. However, it is sharply increased in the cuticle, where it reaches the maximum possible for these formations. The bundle of sensory hairs located above the cuticle has a concentration of protein close to that of the cytoplasm. This concentration is significantly increased in the basal corpuscle located behind the cuticular lamina and in the kinocilia proceeding from it which can be observed with an immersion magnification of a light microscope and identified with the aid of electron optics. The protein content in the cellular membrane is somewhat higher than in the cytoplasm. The nucleus of a receptor cell has a well defined nuclear membrane, differentiated by the high concentration of total protein. The karyoplasm of the nucleus is slightly stained, which indicates a low concentration of total protein contained in it. The nucleolus is rich in protein, standing out clearly against the background of the chromatin which is poor in protein. Around the sharpened base of the receptor cell there are dark sharpened formations rich in protein, connected with the nerve fibers approaching them. It is possible to identify them from the small bud-shaped nerve endings of dendrite neurons of the cochlear ganglion located here.

The cytoplasm of supporting cells is distinguished by the very low concentration of protein, which is somewhat increased in the cellular membranes. At the summit of supporting cells, small desmosomes are visible in which the concentration of protein is elevated to the maximum. Nuclei of supporting cells have a well-defined nuclear membrane with an average concentration of total protein and a light karyoplasm lacking total protein. The nucleolus lying in the center of the nucleus is very rich in protein. The chromatin approximates the nuclear membrane in protein content. On a light background, formed by the cytoplasm of supporting cells, the nerve fibers which pass through here stand out clearly, and are distinguished by an average concentration of protein. They approach the receptor cells and branch in tree fashion near their bases, forming bud-shaped nerve endings on them which are very rich in protein, as was indicated above.

The tectorial membrane lying above the summits of receptor cells is very rich in total protein, although the concentration in it is somewhat less than in the cuticular laminae and in the desmosomes.

Cells of the folds of the Tegmentum vasculosum, with analysis for total protein, are clearly divided into dark and light cells. The dark cells which are located primarily on the periphery of cytoplasm, which is very rich in total protein, show a maximum reaction to protein in their nuclear membrane, chromatin and nucleolus. The cytoplasm of light cells is distinguished by the moderate concentration of protein which in the nucleus significantly increases in the chromatin at the nucleolus, but still yields to the concentration of protein in the nuclei of dark cells.

Neurons of the cochlear ganglion are characterized by a moderate concentration of total protein in their cytoplasm. It is significantly higher in the clumps of Nissl's substance located along the periphery of the cellular body. The concentration of protein in the cytoplasm of individual neurons may vary. The nucleus of a neuron has a moderate concentration of protein in the chromatin and somewhat greater in the nuclear membrane and in the nucleolus, which is irregular in form. However, in the nuclei of individual neurons, primarily in those having lower concentration of protein in the cytoplasm, a low concentration of protein in the nucleolus is observed. A significant concentration of protein is observed in processes of neurons. /38

The distribution of carboxyl groups, in general, corresponds with the distribution of total protein with the only difference being that the concentration of carboxyl groups in the cytoplasm of supporting cells is somewhat higher than that which is characteristic for total protein. In the tectorial membrane in the cells of the Tegmentum vasculosum and in the neurons of the cochlear ganglion, no large difference between the content and location of total protein in carboxyl groups in protein molecules is noted.

Several peculiarities are observed in the content and location of thiol groups in the structures of Corti's organ of birds. Thus in the cytoplasm of receptor cells a high concentration of thiol groups is observed, which is scarcely increased in their particular lamina. There is an imperceptible increase in their concentration, even in the basal corpuscle of the kinocilia and in the kinocilium itself. Karyoplasm of the nucleus of a receptor cell gives a weakly positive reaction to thiol groups, which indicates their insignificant concentration in the karyoplasm, and in general corresponds with their protein and carboxyl group content. A very significant concentration of thiol groups is present in the chromatin and nucleolus of the nuclei; however, the concentration of the thiol groups of the nucleolus is different in individual receptor cells.

In the cytoplasm of supporting cells there is a moderate concentration of thiol groups; however, the difference in their concentration both in the cytoplasm receptor and supporting cells is significantly less sharp than we saw with the reaction to total protein. The fact is interesting that in the cytoplasm of supporting cells of thiol groups form thin fibers which do not appear with the reactions to protein and carboxyl groups of protein molecules and which still cannot be found under the electron microscope. The nuclei of supporting cells are seen with difficulty against the background of the darker cytoplasm due to the low concentration of thiol groups in the nuclear membrane and in the chromatin, which does not greatly exceed their concentration in the cytoplasm. The large concentration of thiol groups is observed in the nucleolus of the nuclei of the supporting cells.

Due to the large content of thiol groups in the cytoplasm of

supporting cells and its fibrous structure, with this reaction the passage of nerve fibers in the receptor epithelium of Corti's organ is less clearly traced. However a sharp increase in thiol groups at the points where the bud-shaped nerve endings are located makes them more noticeable than with reaction to carboxyl groups.

An unusually high concentration of thiol groups is observed in the tectorial membrane whereby significant portions in it belong to the disulfide group. This attests to the high content of sulfur containing amino acids and allows one to assume that processes of denaturing protein with a change in the configuration of its molecules must play a large role in its function.

In the Tegmentum vasculosum, with the reactions of thiol groups, the difference between dark and light cells appears to be very sharp due to the high concentration of thiol groups in the cytoplasm of dark cells and the minimal concentration in the light cells. This causes one to assume that the difference in their chemical content, to a significant degree, is connected with the sulfur-containing amino acids. The nuclei in the dark cells have a content of thiol groups similar to that of the cytoplasm; thus they can be seen only with great effort, and their structure is very poorly pronounced. /39
In the light cells the nuclei are quite visible, but the concentration of thiol groups in their membrane, chromatin and nucleus is average.

In neurons of the cochlear ganglion the cytoplasm of individual neurons varies greatly in concentration of thiol groups. In their nuclei a very swollen nuclear membrane is characteristic, evidently due to the adjacent chromatin. The karyoplasm has a weak content of thiol groups while both in the chromatin and in the nucleolus it is very high. The content of thiol groups of average concentration is observed in the processes of the neuron.

Thus for a receptor epithelium of Corti's organ of birds, large differences in the structural and cytochemical organization of receptor and support elements are characteristic. They are somewhat equal with reaction to thiol groups. The cuticle of receptor cells contains primarily total protein and protein molecules, rich in terminal carboxyl groups, and to a lesser degree sulfur-containing amino acids and protein molecules with open sulfhydryl groups. The tectorial membrane is rich in total protein, carboxyls and chiefly thiol groups of protein molecules and in the Tegmentum vasculosum the difference between the dark and light cells is determined by their protein content and is not connected with RNA. The cytochemical characteristics of neurons of the cochlear ganglion is less different from that of neurons of the vestibular ganglion.

The Structure of the Mammalian Cochlea

The mammalian cochlea is arranged in the shape of a spiral around its own axis, i.e., the modiolus. The latter consists of a

spongy osseous tissue, between the lattice beams of which are located the ganglionic accumulations and the nerve fibers of the spiral ganglion proceeding from them. The vascular supply of the modiolus is characterized by the presence of perivascular sheaths, which evidently damp the expansion and contraction of their inner cavities in relation to the neighboring perilymph (Kolmer, 1927).

From the modiolus a thin osseous lamina, the *Lamina spiralis ossea*, proceeds in the form of a spiral, which in turn consists of two layers in which in the radial canal pass the myelinated dendrites of neurons of the spiral ganglion. The openings for the exit of these dendrites (*Foramen nervosa*) are found at the base of the internal cell column. The upper layer of osseous lamina, the *Habenua perforata*, turns directly into the vestibular lip or spiral limb, the lower layer, thickened near the modiolus into the basilar membrane.

The cochlear canal of the membranous labyrinth, *Ductus cochlearis*, in which Corti's organ is located, is presented as a blindly terminating sac expanded toward the top of the cochlea and turning a number of its helices in a spiral curve. In monotremata there is only half a helix, in placental mammals, their number can reach 4-1/2 (the guinea pig). In man there are 2-1/2 helices. The usual length of the cochlear duct of man is 35 mm.

At the base of the cochlear canal there is an expansion, i.e., the blind sac of the vestibule (*Coecum vestibularis*), which is united with the aid of a narrow canal (*Ductus reuniens*) with the cavity of the sacculus. In cross section the cochlear canal has a triangular form. Three walls are distinguished in it which along the axis are respectively called the superior, external and inferior. The superior wall of the canal is thinner--this is Reissner's membrane, separating it from the vestibular ladder. The external wall is of greater thickness and concave form. It lines the upper half of the spiral connection in the form of the so-called vascular stria and takes part in the formation of endolymph (Held, 1926; Kolmer, ^{/40}1927; Saxen, 1951). The lower wall of the cochlear canal has a more complex structure. It represents a continuation of the *lamina spiralis ossea*, on which the connective tissue intumescence is located, i.e., the spiral lip with the tectorial membrane proceeding from its epithelial and spiral incision, from which the basilar membrane begins with Corti's organ located on its endolymphatic surface. The basilar membrane is attached to the spiral ligaments.

Reissner's membrane is a thin membrane about 2 μ in thickness, which, from the side of the cochlear canal, is covered by a polygonal, flat epithelium with distinct intercellular margins. These cells have an oval nucleus and are very strongly stained with basic stains. According to the data of electron microscopy (Bairati and Iurato, 1964), in the cytoplasm of these cells there are few mitochondria, a small Golgi's apparatus and an endoplasmatic reticulum which consists of vesicles covered with Palade's granules. From the

side of the vestibular ladder Reissner's membrane is lined with a thin endothelium of mesenchymal origin, the cells of which are less differentiated and poor in endoplasmatic organelles. Between these two layers of cells there is a very thin, structureless membrane 200 Å thick. Here in several types of animals (rabbits and goats) blood vessels can be found (Shambaugh, 1905). In man, Reissner's membrane does not contain vessels.

The Vascular Stria. The external wall of the cochlear canal adjacent to the spiral ligaments is lined with epithelium of the vascular stria and epithelium of the external incisure (Sulcus externus), and the so-called external spiral prominence (Prominentia spiralis). The epithelium of the vascular stria has a complex structure. According to the data of light microscopy it consists of low cylindrical cells, located on capillaries and the larger vessels both of the arterial and veinous types (Kolmer, 1927).

According to the data of electron microscopy (Engström, et al., 1955; Smith, 1957; Bairati and Iurato, 1960a; Spoendlin and Balogh Jr., 1963), it was established that the epithelium of the vascular stria consists of basic and transitional cells located at the border with the spiral ligaments. Transition cells have an oblate shape and are located in 4-5 rows. They are bordered more or less equally by a plasmatic membrane. Their cytoplasm is distinguished by low electron density, and it contains few cytoplasmic organelles. Among the basic cells, light deeply situated cells and marginal dark cells, which are very closely bound to each other, are distinguished among the basic cells. The dark cells usually cover the entire surface of the vascular stria and by their long "open-work" cellular processes of irregular form they contact the light cells. By their cellular plasmatic membrane they are separated from the endolymph of the cochlear canal and accumulate near the surface of the vacuoles and secretory granules. Their cytoplasm contains numerous tubular and vesicular formation, which are covered with membranes of the endoplasmatic reticulum, scattered with Palade's granules, a large number of mitochondria, and a very distinct Golgi's apparatus. The nuclei are located either near the surface or are removed into the body of the cell. The structure of these cells is analogous to the *Plana semilunata* cells (Bairati and Iurato, 1960a, 1960b); to renal tubules (Pease, 1955), to nonpigmented epithelium of the ciliar body (Holmberg, 1956); and to the Plexus choriodei (Maxwell and Pease, 1956). This permitted Bairati and Iurato (1964) to express the hypothesis that the cells of the vascular stria have a similar function and ensure the filtration of endolymph and the regulation of ion concentration.

The marginal plasmatic membrane forms numerous folds, which surround the walls of the capillaries and create the illusion of their intercellular dispersion. However, electron microscope investigations show that there is always a cellular cytoplasmic membrane between the wall of the capillary and the cytoplasm of the cell, and thus the capillaries in the vascular stria are always

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situated extracellularly. In the narrow portions of cytoplasm located between the folds of the plasmatic membrane there are long rows of mitochondria, vesicles and free Palade's granules. These cells contain grains of pigment, i.e., melanin, which consists of individual grains or of accumulations of them. The formation of pigment takes place in the region of Golgi's apparatus (Güttes, 1958; Reiser, 1959) and in Beck's opinion (1961), the pigment which is found in the vascular stria may take part in the exchange of substances of the cochlear canal. These cells are differentiated by their different heights, which determine the irregularity of the surface of the vascular stria.

Endolymph and perilymph. The endolymph fills the cochlear duct and the other parts of the labyrinth including the Ductus and Saccus endolymphaticus. This latter lies above the brain between the lamina of the dura matter, near the membranous venous sinus. The endolymph enters the space of the labyrinth and leaves its bounds in definite parts, for example, in the Saccus endolymphaticus and in the vascular stria of the cochlea and in analogous structures of the auditory spots and the cristae of the vestibule.

The epithelium of the external incisure, lying between the vascular stria and the basilar membrane provides a series of cytoplasmatic processes in the direction toward the blood and lymphatic vessels, distributed in the connective tissue of the spiral ligaments. The latter display an ability for phagocytosis, swallowing detritus, which is always present in the endolymph. Several cells also reveal phenomenon of degeneration and pycnosis of the nuclei. At the present time it is accepted that an outflow of endolymph exists in the region of external incisure, although it is impossible with histological methods. The epithelium of the external incisure turns into several types of Claudius' supporting cells of an analogous structure.

The basilar membrane (Membrana basilaris) is the fundamental wall of the cochlear canal on which the elements of Corti's organ are located (Papilla basilaris). Its surface turns toward the perilymph of the tympanic ladder, is covered with a layer of thin endothelium of mesenchymal nature, under which vessels can be located, which are always absent in Corti's organ itself. It is a connective tissue lamina which stretches along the entire cochlea canal in the form of a spiral. Its external edge begins in the form of separate fibers of the Hebenula perforata, in which the Lamina spiralis ossea terminates. In the basilar membrane two portions are differentiated, which turn into one another: the exterior zone, the Zona arcuata on which the tunnel is located, and the internal and external hair cells of Corti's organ; in the region of the base of the external columns it turns into the external zone, the Zona pectinata, on which basically Hensen's cells are located. The external zone is attached to the spiral ligament. According to Neubert's anatomical data (Neubert, 1949), the basilar membrane not only extends along the cochlea but is expanded. Its breadth fluctuates from 0.423 to

0.651 mm.

At the base of the basilar membrane structure lie thin collagen fibers, i.e., "strings", which stretch in the form of a continuous radial bundle, directed perpendicular to its length. The fibers located in the region of the Zona arcuata, are distinguished by their thinness and tenderness. On the other hand, the fibers of the Zona pectinata are thicker and straighter. The fibers become broader and longer, the higher they go toward the summit of the cochlea. The shortest fibers are located in the basilar membrane at the level of the Coecum vestibularae; here their length is equal to $104\ \mu$, and at the summit of the cochlea they equal $504\ \mu$ (Kolmer, 1927; Bargmann, 1951). The fibers are located in the fundamental homogeneous interstitial substance. According to Retzius, the total number of fibers of the basilar membrane reaches 24,000 (Retzius, 1884). /42

M.D. Labdovskiy (1874) first turned attention to the fact that in the Zona pectinata the fibers are located in two rows and between them there is a transparent layer of fundamental interstitial substance. In the Zona arcuata, according to his data, there is only one layer of fibers.

According to electron microscopic investigations (Engström, 1955a; Iurato, 1962a, 1962b), the basilar membrane consists of four layers. The upper support layer directly adjoins the epithelium of Corti's organ. On the surface of the support layer is formed a homogeneous and continuous membrane of average electron density, approximately $200\ \text{\AA}$ thick, separated from its own plasmatic membrane of the epithelium of Corgi's organ by a fissure $180\ \text{\AA}$ in width. Directly under the membrane is the first fibrillar layer consisting of fibers of $85\text{--}105\ \text{\AA}$, surrounded by a homogeneous basic substance. After them, there follows the upper homogeneous layer, directly turning into the lower fibrillar layer contacting with the endothelial cells, lining the tympanic surface which Iurato calls the cells of the basilar membrane. In the first fibrillar layer are located fibrils of an average size ($0.2\text{--}0.4\ \mu$ in diameter), which, on the average, in the basal helices are not very distinctly separated from one another and form numerous anastomoses, creating the impression of a continuity of fibrillar layers. The second fibrillar layer consists of large fibrils (diameter $0.5\text{--}1.5\ \mu$) of the regular cylindrical form, which are distinctly separated from one another by homogeneous substance. The space between the two fibrillar layers is filled with a homogeneous basic substance in which, in the middle and basal helices, are located the numerous small and average size fibrils. The lower homogeneous layer lacks fibrils. The so-called cells of the basilar membrane (or endothelial cells) lining the tympanic surface have a cytoplasm poor in organelles, consisting basically of a hyaloplasmatic matrix with low electron density. The Zona arcuata and Zona pectinata of the basilar membrane reveal a significant difference. In the region of the Zona arcuata there is a layer of radial fibers distributed and anastomosing between each other, forming together a net-like structure lying in a homogeneous trans-

parent mass. These net-like fibers, however, lie close to the base of the cell column but never enter them. On the tympanic side of the basilar membrane under the fibrils there is a thin layer of homogeneous material on which the epithelium and capillaries are located.

From the base of the exterior cell columns of Corti's organ, the fibrillar layer of the basilar membrane divides into two layers which stretch along the entire Zona pectinata. They are separated from one another by a transparent formless layer. The upper of these layers is located directly under the plasmatic membrane of elements of Corti's organs. In both layers the fibrils are numerous, their diameters equal 300 Å. The large fibrils anastomose one another, but these anastomoses are thin. At a point of transition into the spiral ligament both fibrous layers are fused. It is necessary to mention that in the lower layers these fibrils form a continuous membrane which is difficult to see under the light microscope but which, nevertheless, was discovered first 90 years ago by M.D. Lavdovskiy (1874).

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Thus the basilar membrane basically consists of four layers: (1) the upper fibrous layer on which Corti's organ is located; (2) the upper homogeneous layer; (3) the lower fibrous layer; (4) the lower homogeneous on which the endothelium lining the tympanic surface is located.

Diffractionographic and chemical investigations show that the fibrillar portions of the basilar membrane consist of a protein-like substance which differs from collagen and from elastic fibers (Held, 1926; Engström, 1951), and possess several characteristics of a protein of the KMEF-group (Keratin-myosin-epidermin-fibrinogen; cf. Iurato, 1962a). The organization of such a fiber was studied under a polarized microscope. In addition, bundles of fibers with double refraction were found, which variously pass into the different sections of the basilar membrane (Iurato, 1957, 1962a). In the region of the spiral limb there are fibers proceeding superficially in the region of the auditory denticles of Huschke and fibers which are deeply located lying parallel to the spiral. In the region of the basilar membrane the fibrous structures are located radially between the tympanic lip of the spiral limb and the basilar crista of the spiral ligament. Bairati and Iurato (1964) found two chief layers and between them a network of small, thin bundles of fibers of different directions, which intersect with one another.

Corti's organ (Papilla basilaris) is located on the vestibular surface of the basilar membrane. In cross section the organ has the front of a trapezoidal fold, in the middle of which there is a tunnel formed by shallow columns and nearer the surface Nuel's space filled with endolymph. Nuel's space is bounded on one side by the reticular formation and on the other side by the bases of the external hair cells of the first series and parts of Deiter's cells abutting them; from the third side it is bounded by the external

surface of the exterior cell columns. Held (1926) proposed naming Nuel's space the exterior tunnel, with which certainly it is possible to agree. Along the bottom of the tunnel and Nuel's space pass the dendrites of the neurons of the spiral ganglion which are deprived of myelinated membranes, directed toward the bases of the internal and external hair cells.

A connective tissue intumescence abuts Corti's organ from the interior side, which is located on the laminar spiralis ossea before the point of nerve fiber exit, the so-called vestibular lip (*Labium vestibularae*), which at the point of formation of the basilar membrane turns into a depression called the internal spiral sulcus (*Sulcus spiralis internus*). The tectorial membrane proceeds from a sharp angle of the lip, which is superimposed from above onto elements of Corti's organ including the hair cells.

The surface of the vestibular lip, the so-called spiral lip, is covered with a smooth epithelium of unique construction. Its elements are located in parallel rows, directed along the radius of the cochlea. The portions of the cells containing the nuclei are immersed deep in the underlying connective tissue so that a unique mosaic of their surfaces lies on the surface of the lip. This pattern gave a basis for Huschke (1835) and Corti (1851) to isolate elements of the spiral lip as "auditory denticles". Our own observations with the aid of histochemical methods reveal the unquestionable presence of secretory activity in these elements and, in particular, the participation of their nuclei in these processes (Vinnikov and Titova, 1958a). Certainly they cannot be connected with the appearance and function of the tectorial membrane, which evidently is a structure formed by epithelium of the vestibular (spiral) lip. Such a concept corresponds with the data of autoradiography of the epithelial covering of the spiral lip (Belanger, 1953; Frieberg and Ringertz, 1956). Radioactive sulfur (S^{35}) very quickly penetrates the tectorial membrane, evidently due to the intensive activity of the epithelium of the vestibular lip. /44

As far as the lining of the spiral sulcus is concerned, it appears to be several rows of large smooth polygonal cells with large oval nuclei appearing as a continuation of elements covering the spiral lip. On the spiral surface of these cells, submicroscopic villi were revealed with the aid of an electron microscope (Spoendlin, 1957; Engström and Wersäll, 1953b). These cells turn directly into a series of elements appearing as supporting cells for the internal hair cells. These latter are covered almost to their summit by epithelial cells of the spiral sulcus superimposed upon them in an embryocated fashion.

The Tectorial Membrane (*Membrana tectoria*) is basically connected with the epithelium of the vestibular lip. Its structure was investigated by a number of authors (Hensen, 1863; Lowenberg, 1864; Retzius, 1884; Ayers, 1891; Coyne and Cannieu, 1895; Exner, 1897; Czinner and Hammerschlag, 1898; Rickenbacher, 1901; Held, 1902, 1909;

Kishi, 1907; Shambaugh, 1907; Hardesty, 1908, 1915; Prentiss, 1913; Kolmer, 1927; Borghesan, 1949, 1952, 1954, 1957, 1959; Mygind, 1952; Hilding, 1952, 1953a, 1953b; Smith, 1955; Wersäll, 1956; Bairati, Iurato and Perniss, 1957; Iurato, 1957, 1960, 1962a; Bairati and Iurato, 1964). It is a tenioid lamina of gelatinous consistency, which stretches in the form of a spiral along the entire length of Corti's organ and increases both in width and in thickness from the base to the summit. In man its width at the base of the cochlea equals 240 μ ; at the summit, 400 μ . In the rat it is, respectively, 100 and 290 μ (Held, 1926; Iurato, 1957, 1961).

Along the radius of the tectorial membrane, according to the data of Held (1926), by investigation of a living membrane under a phase-contrast instrument (de Vries, 1949; Iurato, 1960), it is possible to subdivide it into three parts: (1) the axial part which is intimately connected with the summit of the marginal epithelial cells of the tibular lip consisting of thin fibrillar structures; (2) the middle or main part lying freely above the epithelium of Corti's organ with its internal and external hair cells having a radially distributed fibrous structure with a slope angle of 30° (Hilding, 1952); (3) the lateral or external (marginal) portion located above the cells of Hensen and Claudius, revealing a reticular structure formed of 6-7 fibers passing parallel to the free edge of the membrane and radial fibers intersecting with them. Between the fibers there is a transparent sticky substance. On the marginal portion the fibers become cruder and more intensively stained. They are connected with each other by a means of ponticuli and anastomoses. On the surface of the tectorial membrane vagal elements are often discovered (Vinnikov and Titova, 1957).

The submicroscopic structure of the tectorial membrane (Iurato, 1960, 1962a; Bairati and Iurato, 1964) consists of nonanastomosing fibers, i.e., protofibrils with a diameter of 96 Å, having nonperiodic structure. These protofibrils are included in a formless substance in which they are located in groups and under light microscopy, in particular of living tissue under phase contrast apparatus they appear as fibrous structures. /45

Chemical and diffractographic investigations (Bairati, et al., 1957; Iurato, 1960) showed that the tectorial membrane consists chiefly of protein of the soft carotin groups with an insignificant content of cystine. They prove the incorrectness of the hypothesis concerning its collagen nature which was confirmed by histochemical data and investigation in polarized light (Rudall, 1947; Reed and Rudall, 1948; Randall et al., 1952).

The epithelium of Corti's organ lies between the Sulcus spiralis internus and externus on the vestibular side of the basilar membrane from which it is divided by a homogeneous layer 200 Å in width. The epithelium consists of three types of cells: (1) receptor, (2) supporting cells with fibrillar structure (internal supporting cells, cell columns, Deiter's cells) and (3) little differentiated support-

ing cells (Hensen's cells and Claudius' cells).

Receptor cells of Corti's organ are represented by internal and external hair cells which are distinguished from one another not only by their location, but also by their structure and innervation which is undoubtedly connected with the functional characteristic.

Internal hair cells have an irregular pitcher-shaped form in longitudinal section with a curved neck and with an expanded base of an irregular oval shape. In cross section they seem to be polygonal (Bairati and Iurato, 1964). The internal hair cells are located in one row between the internal supporting cell phalanges and are bounded by high cylindrical cells of the internal sulcus near the summits of the internal cell columns; however, they do not have any special support apparatus. At the point where they touch the neighboring elements there are well-defined intercellular fissures. The axis of the internal hair cells is inclined toward the basal membrane and forms an obtuse angle of 140° against the modiolus, repeating the angle of inclination of the internal cell column. If we look at Corti's organ from above, then the internal hair cells appear to be lying in a horizontal position with a slightly elevated summit, rising above the level of the cuticular edge. Their slightly convex summit bears 34-40 hairs on their surface. On each cell they are located in two parallel rows (Engström, 1951; Iurato, 1961). The number of hairs fluctuates depending on the position of the cells on one helix or another and upon the type of animal (Kolmer, 1927; Engström, 1951). According to the data of Held (1926), the number of hairs on the internal hair cells amounts to 41-44 in the basal helix of man, 64 on the second helix, while Kolmer (1927) counted 30-40 hairs. Iurato (1961) gives the number of hairs on the basal helix on the cochlea of the rat as 34 ± 4 hairs per cell and 41 ± 5 per cell on the summit and for the second helix of the guinea pig, 34-40.

The cytoplasm of cells, under the condition of fixation by means of filling the vessels, is poorly preserved due to the absence of vessels in Corti's organ itself (Kolmer, 1927). The large round nucleus is located at the base of the cell. The number of internal hair cells is significantly less than that of external hair cells. In man there are approximately 3500 (Engström, 1958); in the cat, 2600; in the rabbit, 1600.

Numerous electron microscope investigations (Engström, 1951; /46 Katsuki and Covell, 1953; Wersäll, 1956; Friedmann, 1959a, 1959b, 1962, 1963; Engström and Wersäll, 1953a, 1953b, 1953c, 1958a, 1958b; Engström et al., 1953; Spoendlin, 1957; Engström, Ades and Hawkins Jr., 1962; Iurato, 1961; Bairati and Iurato, 1964; Wersäll, Hilding and Lundquist, 1961, and others), showed that both the external and internal hair cells are constructed on the same general plane. They are surrounded by a double marginal protoplasmic membrane 70 Å thick, and their cytoplasm has a different structure in zones lying at different depths.

The cuticular lamina located at the apical end of an internal hair cell is of the form of an elongated oval, with a long axis, parallel to the axis of the external tunnel (Engström, Ades and Hawkins Jr., 1962). It consists of electron dense granulated material. In each cell there is a narrow portion free of cuticle in which the basal corpuscle is located (Engström, Ades and Hawkins Jr., 1962). In mammals the receptor cells lack kinocilia. At the summit of the cells are located immobile hairs, the stereocilia, which have a free supercellular portion and a thin root portion attached to the cuticle. The root of a hair is slightly narrowed downward and often reaches the cytoplasm. Around the cuticle is the narrow part of the hair or the neck, the diameter of which does not exceed 500 Å. The hair itself has a length of around 4.5-5 µ. It proceeds from the free surface of the hair cells to the inner surface of the tectorial membrane. The sharpened hair which lowers downward forms a tube-like structure with slightly dark walls. In the center of the tubules dark thin fibrils are seen. The tubule extends upwards to 2/3 the length of the hair forming a complete central core, the so-called axial fibril (Engström, Ades, and Hawkins Jr., 1962). The stereocilia of an internal hair cell is twice as thick as that of an external one and has a diameter of 0.35 µ (Iurato, 1961). The axial fibril is surrounded by a less dense substance than the substance of the cuticle. The thin fibrillar substance of a hair is oriented parallel to its long axis (Iurato, 1961; Wersäll, Hilding and Lundquist, 1961). The external surface of a hair is covered by an external membrane having the same structure as the plasmatic membrane of the cell, of which it is a dilation (Iurato, 1961) and into which it is directly transformed. Inside this membrane at a distance of 70 Å is a second membrane continuous with the root portion of the hair. Thus each immobile hair is surrounded by two membranes of different origin and attachment (Engström, Ades and Hawkins Jr., 1962).

The cytoplasm of an internal hair cell is richer in cytoplasmic organoids and is more osmophilic than the cytoplasm of an external hair cell. It is darker and a more distinct structure than an external hair cell.

The apical supranuclear portion of an external hair cell has a finely granulated cytoplasm in which there is a large number of round or oval mitochondria in granules. The mitochondria are located directly under the cuticle. No clear accumulation of them in the internal surface of the vertical wall of the cell is observed (Engström and Wersäll, 1958a). Hensen's corpuscle in the internal hair cells is absent. The cytoplasm of the apical portion is rich in dark osmophilic granules of various density with a diameter of 0.2-0.4 µ (Engström and Wersäll, 1958a; Iurato, 1961). The vertical (lateral) wall of an internal hair cell is delimited from the surrounding elements by a clear plasmatic membrane under which membranous formations are scattered (Engström, 1960). In the region of Golgi's apparatus cisterns of the endoplasmic reticulum are weakly developed.

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The nucleus is located closer to the base in an expanded portion of the cell. It is distinguished by its round form and significantly larger diameter than an external hair cell.

In the subnuclear portions of an internal hair cell, directly below the nucleus and near it, there is a small number of α -cisterns, bordered by granulated membranes, which open in crack-like apertures into the cytoplasm (Engström and Wersäll, 1958b). They are usually located parallel to the nuclear membrane (Wersäll, Hilding and Lundquist, 1961). These membranes were described by Wersäll (1956), and Engström (1960a) in Type I receptor cells of the vestibular apparatus. Similar membranes are usually found in cells differing by their increase in protein metabolism (Wersäll, Hilding, and Lundquist, 1961). Under these membranes in a thinly granulated cytoplasm are scattered round or oval mitochondria which never form parallel rows.

On the internal hair cells the endings of hair cells are grouped chiefly at the base of the cell, but a certain number of small and smooth nerve buds are usually located on the lateral surface of the cell. The basic group of sensory endings consists of individual, nonmyelinated axons, between which there are narrow endings. Friedmann (1963) considers that only one form of nerve ending terminates on the internal hair cell. Iurato (1961) bypasses the nature and structure of nerve endings on the internal hair cells without comment, only mentioning that nerve endings come into close contact with the basal surface of the cellular membrane which is separated from the plasmatic membrane of the nerve ending by a synaptic fissure 150 Å in width. Wersäll, Hilding and Lundquist (1961) note that nerve endings are located very unequally on the entire base of an internal hair cell and are connected with nerves of the internal spiral plexus. Several of these nerve endings are filled with small vesicles; others are light and do not have vesicles. In these endings it is possible to see separately large mitochondria. The plasmatic membrane of each nerve ending is separated from the plasmatic membrane of the hair cell by a thin synaptic tissue. Judging by the given electronograms, the conclusion of the authors on the presence of two types of nerve endings on the base of an internal hair cell seems to us to be very probable. The presence of granulated and agranulated nerve endings on the internal hair cells is described by Engström and Wersäll (1958a, 1958b).

External hair cells are shaped like elongated cylinders with round lower end and smooth upper surface, located inside the reticular membrane. By their lower ends these cells are located in special depressions on the upper surface of Deiter's cells. A round cellular nucleus is found on the lower portion of the cell. The upper surface of the cell is delimited by a clear and thick cuticle from which proceed various numbers of thin hairs projecting upwards. The number of hairs on an external hair cell differs among the various types and changes depending on the distribution of cells on one helix or another of the cochlea. Kolmer (1927) considers that in

the most primitive mammals there are around 8-12 hairs on each cell; Held found 100 hairs on each cell in man; Iurato found 75 ± 6 in the basal helix of the rat, in the middle helix 96 ± 5 , and in the upper 62 ± 8 . In the guinea pig, rat and monkey the stereocilia are located in the form of a letter W and they are calculated at 115 per each cell for the middle helix (Engström, Ades and Hawkins Jr., 1962). They stretch from the surface of the cell to the inner surface of the tectorial membrane. The number of external hair cells in man is 12,000 (Retzius, 1884); in the cat 9900; in the rabbit 6100-6200 (Retzius, 1884); Bargmann, 1951). They are located in 3-4 rows lateral of Nuel's space. In several anthropoids and in man, the number of rows of the external hair cells in the upper helices of Corti's organ can be increased to 4-5 (Kolmer, 1927). External hair cells, located near the base of the cochlea have longer hairs than cells located near the summit. The difference in their length is $2.5-5 \mu$ in the rat (Engström, Ades, and Hawkins Jr., 1962). Kolmer considers that on each external hair cell the hairs regularly change in height; the highest is located near the basal corpuscle (Kolmer, 1927). The upper end of the external hair cells are connected with the digitate processes of Deiter's cells (phalanges). However, the main part of an external hair cell is free and completely surrounded by corticolymph (Engström, 1960). The axis of an external hair cell forms a 45° angle with the basilar membrane. Thus, the long axes of an internal and external hair cell form almost a right angle (Bairati and Iurato, 1964). The upper surface of each external hair cell is covered with cuticle, which is relatively smooth on the upper surface and on the lower surface forms a crest and projects into the cellular cytoplasm. Under the electron microscope the cuticle is dark and granulated (Engström and Wersäll, 1958a, 1958b). The cuticular lamina forms a characteristic angle around $20-25^\circ$ with the long axis of the cell (Engström, 1960). The cuticle gradually swells in the direction toward the modiolus. Under the space of the cellular surface which is free from cuticle is located the basal corpuscle (Engström, Ades and Hawkins, Jr., 1962; Flock, 1964). and a large number of cytoplasmic organelles. The form and size of the cuticular lamina is different on different helices and rows of external hair cells. On the surface of the cuticle there is a various number of hair-like processes (macrovilli or stereocilii), which have intracellular or cuticular roots, but the kinocilia is absent from them (Friedmann, 1962). Each hair has a double membrane on its surface. The exterior of these turns directly into the cytoplasmic membrane of the cell, while the internal is directly connected with the root structure of the hair. Engström proposes in his 1960 paper that the various points of attachment indicate that the bending of the hairs elicits cutting forces between two membranes. However, in his study with Ades and Hawkins (Engström, Ades and Hawkins Jr., 1962) he departs from this view and indicates that at the present time there are no bases for considering that the fundamental stimulation of the cochlear hair cells is elicited by bending of the hairs. As the result of electron microscope and histophysiological investigation of the sensory hairs of receptor cells of the inner ear,

Engström and colleagues come to the conclusion that the rigid stereocilia of external hair cells act as levers, passively transmitting energy of cutting motions of the tectorial membrane to the cuticular lamina and through it to the basal corpuscle and the organelles accompanying it. These authors conclude that the basal corpuscle representing the kinocilia vestibular hair cells may be considered as the basic exciting structure of the cochlear hair cells (Engström, Ades and Hawkins Jr., 1962).

The stereocilia of external hair cells has the same structure as the stereocilia of internal hair cells but are significantly thinner than the latter. The diameter of external hair cells is 0.15μ ; i.e., less than half the size of the internal (0.35). According to the data of Borghesan (1952, 1954, 1957, 1959), the stereocilia of hair cells turns directly into fibers of the tectorial membrane. This concept was refuted by Iurato (Iurato, 1960) who indicated that the double membrane of macrovilli (stereocilia) forms a clear, definitive border separating the hairs from the tectorial membrane. However Engström and Wersäll (1958b) consider that it is impossible to conclusively decide this question with the aid of an electron microscope, since it is impossible to receive both ends of the stereocilia on one ultra-thin section. Moreover, they note that they could never succeed in observing a direct transition of stereocilia into the tectorial membrane. /49

Even the distribution of stereocilia on external hair cells in the form of the letter W with the gradual elevation toward the modiolus deserves attention. Engström and coauthors (Engström, Ades and Hawkins Jr., 1962) expressed the hypothesis that the W-shaped distribution of hairs on external hair cells is an adaptation increasing the sensitivity to definition of direction of motion.

The intracuticular part of the hair cell is provided with an accumulation of narrow long mitochondria and granules. These osmophilic or dense granules are significantly increased in size when a sound of great intensity and duration acts upon the cochlea (Spoendlin, 1958, 1959; Engström, 1960). The cytoplasmic organelles are clearly concentrated under the thin portion of the cuticular lamina.

Below the subcuticular portion is located a round laminar formation, Hensen's corpuscle (Hensen, 1863; Held, 1926), and electron microscopy shows that this corpuscle has a round center surrounded on the periphery by one or several dense membranes in 5-7 rows of interrupted membranes (Engström, 1960) $50 \text{ \AA}$ thick, with a distance between them of $100 \text{ \AA}$ (Spoendlin, 1957). Outside of these membranes are located mitochondria. Engström and Wersäll (1958a, 1958b) indicate that in the external hair cells Hensen's corpuscle, with its continuous laminar structures, is very similar to the usual net of Golgi's apparatus. They consider it possible that Hensen's corpuscle is a specialized or modified Golgi's apparatus. According to their observations, the structures of Hensen's corpuscle are increased in size and number with the action of sounds of high inten-

sity and frequency (2000-3000 Hz for 24 hours) on a guinea pig.

The longitudinal or vertical side of a hair cell is surrounded from the periphery by a plasmatic membrane; on the internal sides of this membrane there are 6-7 layers of the so-called interruptive membranes which have a thickness of around 300 Å (Engström, 1955b). They have a strong similarity to membranes of Hensen's corpuscle (Engström, 1960). Spoendlin (1957) considered that there are here 6-12 separate dark membranes of a thickness of 70 Å and with intervals between them of 100-150 Å. Near the internal surface of this membranous layer is located one layer of longitudinally oriented twisted mitochondria.

At the level of the nucleus the interrupted membranes decrease in number, and in the subnuclear there is usually only one layer which never reaches the region of the nerve ending. The plasmatic membrane and the layer of interrupted membranes surround the central cytoplasmic portion of the supranuclear zone. The cytoplasm here /50 has a low electron density, is unusually homogeneous and contains individually axially located mitochondria (Engström, 1960). According to the data of Spoendlin (1957, 1959), these membranes uninterruptedly turn into the exterior cellular plasmatic membranes. This observation was confirmed by Friedmann (1962) with intravital observation of fixed tissue of the cochlea. Engström (1955b) compares these membranes (lamina) with the pile of laminar discs first described by Sjöstrand (1953) in an external segment of the rods and cones of the retina of the guinea pig. Friedmann assumes that they may participate in the production of cochlear microphonic potential (Freidmann, 1962).

The nucleus of external hair cells is round or slightly elongated with the long axis parallel to the long axis of the cell body. It has a moderately dense karyoplasm containing granules of 140-150 Å in diameter. The chromatin net forms a peripheral accumulation of granules. The nucleolus has a denser and more homogeneous structure. The perinuclear zone of external hair cells is characterized by an intensely hydrated hyaloplasm, in which vesicles of the endoplasmic reticulum are absent. Here there is located usually, a well-developed Golgi's apparatus consisting of smooth-walled canals of vesicles and granules or droplets bearing a secretory function.

Under the nucleus is located only a small number of mitochondria and granulated endoplasmic membranes, which may be compared with the Retzius' corpuscle visible under the light microscope (Engström and Wersäll, 1953a; Smith, 1955). Its dimensions are also increased under acoustic influences (Engström and Wersäll, 1958a, 1958b). In the cytoplasm of external hair cells there is an abundance of free equally distributed ribosomes.

Nerve endings of the external hair cells form a high, morphological specialization. Two types of nerve endings are found here (Engström and Wersäll, 1953a; Engstrom, 1958; Spoendlin, 1957, 1959;

Smith and Sjöstrand, 1961). Engström (1958) showed that there are large and small nerve endings. Large nerve endings are filled with small vesicles of 200-400 Å in diameter, the so-called synaptic granules (de Robertis and Bennett, 1954; Palade and Palay, 1954; Palay, 1956; de Robertis, 1958). In the part abutting the base of an external hair cell they have a two-layered synaptic membrane. At a certain distance from it lie the numerous small mitochondria. Small nerve endings contain larger granules between which mitochondria are scattered in a random fashion. The synaptic membrane of external hair cells in a synapse with small nerve endings has a simple structure. Wersäll, Hilding and Lundquist (1961) found a synaptic bundle, covered with synaptic vesicles, located on the internal side of a simple synaptic membrane of the hair cell, at the point of synapse with a small nerve ending.

Under the base of each external hair cell there are 3-5 bud-shaped nerve endings, which often are distinguished by their size in various nuclei of hair cells. Along the external (third) row of external hair cells they are located in a more isolated fashion and rarely reach the size of those in the first row. In the first row of external hair cells on all the helices of the cochlea the nerve endings form very large bodies of nerve terminals. On the basal helix there are many large and small nerve endings even in other rows of other external hair cells. Smaller and smaller nerve endings are observed in the second and third rows of external hair cells; here basically are found small nerve endings. Under an electron microscope it is clearly seen that the large and small nerve endings under various hair cells have dissimilar structure. It is often observed that the central nerve endings contain significantly darker and more densely distributed mitochondria, and endings located on the external side have lower electron density. Nerve endings located on the medial side are rich in cytoplasm of high electron density. /51

With the aid of the light microscope, internal and external spiral plexuses of nerve fibers were described, lying in the external and internal hair cells, respectively (Held, 1926; Poljak, 1946, et al.). Originally both plexuses were viewed as afferents beginning from neurons of the spiral ganglion of the cochlea. However, the works of Rasmussen (1946) showed that part of the fibers of the spiral plexi are efferents and begin in the olives, i.e., Rasmussen's olivocochlear bundle. An analogous bundle was described in the cochlea of birds and reptiles (Boord, 1961).

Differences in the structure of these two types of nerve endings manifest even a difference in their function. Engström (1958) considers the agranular nerve endings to be afferent and the intensely granulated nerve endings to be efferent. This was confirmed by experiments with severing the so-called Rasmussen's olivocochlear bundle, which elicited degenerative changes in the intensely granulated nerve endings (Iurato, 1961) and the appearance of ACE in the region of the large nerve ending (Wersäll, Hilding and Lundquist, 1961). The statement that efferent nerve endings are connected by

means of synopsis of the hair cells of Corti's organ corresponds to investigations of Fex (1959, 1962) and Desmedt and Monaco (1960). These authors found that efferent fibers not only influence action potentials (AP), but also the cochlear microsome (CM) and summation potentials (SP).

Supporting cells of Corti's organ, in contrast to receptor elements, are always located with their basal ends directly on the basilar membranes in such a way that the hair (receptor) cells are protected from direct oscillation of the latter. The presence of large intracellular fissures, the tunnel and Nuel's space between supporting cells deserves attention. They improve the conditions for the exchange of substances in Corti's organ which is deprived of blood vessels.

Nuclei of the internal hair cells are distinguished by their quite large measurements and comparative poverty of DNA. The largest 1-2 grains of irregular annular formations are located around the nucleoli. On gallocyanine preparations also the general features of the cell are seen, its cytoplasm and nuclear membrane. Lumps and grains of DNA are observed as intensively blue. Also "full" nucleoli after the processing of ribonuclease are stained in a blue color. As far as RNA is concerned, in the internal hair cells it is revealed in equal concentrations in the apical portion of the cells above the nucleus and in the shape of more or less regular, rod-shaped streaks, oriented along the long axis of the cellular body. The nucleus evidently also contains diffuse RNA, since after treatment by ribonuclease its background is significantly more transparent. In addition, the nucleoli are somewhat decreased in size.

The nuclei of external hair cells are somewhat richer in DNA. /52
It is located in the form of grains or clumps in the central portion of the nucleus, where there are also 2-3 larger nucleoli. RNA in the nuclei of external hair cells is located inside the nucleoli and slightly stains the entire karyoplasm. The cytoplasm of external hair cells contains RNA in the form of thin striated red-like structures, located above the nucleus and having a more or less direct orientation along the long axis of the cell. Continuous staining of external hair cells in the form of diffuse blue color after treatment with gallocyanine is not observed. A significant DNA content is found in the nuclei of supporting cells of Corti's organ. In particular, clumps of nuclei of the epithelium of the spiral lip are saturated with DNA. Patterns of distinct nuclear secretion are present here. Nuclei of Hensen's cells, Claudius' cells and the epithelium of the vascular stria are also distinguished by their abundance of DNA. There is somewhat less in the nuclei of cell columns and Deiter's cells. In the cochlea of animals under conditions of relative rest, the more or less equal distribution of general protein is clearly traced in all structures of Corti's organ.

Internal hair cells are distinguished by their fairly rich protein content. It is clearly apparent in the nuclear membrane and

in the cellular membrane. In the nucleus, protein is concentrated in the nucleolus; in the chromatin, it is in the cytoplasm in the numerous granules. Frequently at the base of the hair cells around the nucleus there is a large accumulation of granules having a regular round shape which are distinguished by their significant protein content. In particular, an intensive protein content is observed in hairs of the internal hair cells. Located on the surface of the latter, they form an irregular brown border which extends along the entire spiral of the cochlea. In the external hair cells, there is also a significant concentration of total protein which is somewhat weaker in comparison with that of the internal hair cells. In particular, its concentration in the nuclear membrane, nucleos and chromatin is prominent. In the cytoplasm, protein is concentrated in small granules. Just as in internal hair cells, there is an especially high concentration of protein in the hairs of external hair cells. They have the form of a V-shaped brush intensively stained in a lilac-brown color. An acute angle at the base of this brush is turned toward Hensen's cells.

Finally, it is necessary also to deal with the characteristics of protein distribution in the nerve cells of the spiral ganglion. In connection with the high concentration of protein, the body of the neuron is sharply striated. The nuclear membrane is also sharply striated. The nucleus contains protein in the nucleolus and chromatin. In the cytoplasm, aside from the general diffuse localization of protein, its content is especially high in Nissl's clumps. The concentration of protein is sharply lowered in the axon. In dendrites, on the other hand, it is quite high; therefore they are clearly traced along the entire Habenula perforata.

Distribution of SH-groups in control cochleas somewhat differs from the distribution of general protein. On the external hair cells, the concentration of SH-groups is weaker in comparison with the internal ones. The same SH-groups in the external hair cells are easily traced in the cellular membrane, in the nuclear membrane, in the nucleoli and chromatin of the nucleus and also in the vacuolized cytoplasm. The highest concentration of SH-groups must be noted in the hairs.

In practically all structures of Corti's organ containing SH-groups, SH + SS-groups are found everywhere. The most intensive increase in crimson-colored stain is observed in nerve cells of the spiral ganglion, to which SH + SS-groups give a clearer crimson color in comparison with the treatment of only SH-groups on some. SH and SS-groups are distinguished also by the high concentration of satellites surrounding the nerve cell. /53

In internal hair cells, COOH-groups are represented in a comparatively small concentration in their membranes, cytoplasmic granules, nuclear membrane, chromatin and nucleolus of the nucleus. Their concentration significantly increases in hairs of internal hair cells. External hair cells are also not distinguished by nu-

merous COOH-groups. All their membranes, cytoplasmic granules, nuclear membranes, nucleolus and clumps of chromatin are stained quite intensively in a bluish-red color, which attests to the presence of COOH-groups in them. But an especially significant concentration of COOH-groups is found in hairs of the hair cells.

In neurons of the spiral ganglion, the concentration of COOH-groups is very insignificant. However, they are stained a blue color which increases in the cytoplasm in the regions of Nissl's substance, in the nuclear membrane, in the nucleolus and chromatin. COOH-groups are discovered also in the axons. In dendrites of the region of the Habenula perforata they are not found.

The distribution of glycogen in Corti's organ somewhat differed depending on the type of animal. In the guinea pig, glycogen is found only in the external hair cells along the entire length of Corti's organ and on helices of the cochlea (Vinnikov and Titova, 1961).

The cochlear (spiral) ganglion in man, according to the data of Guild and coauthors (1931), consists of 23,000 to 28,000 bipolar nerve cells, which are more densely located in the second half of the basal and first half of the middle helix (1076-971 cells per 1 mm of length of Corti's organ) and less densely at the beginning and in the apical helix (502 cells per 1 mm of length of Corti's organ; cf. Guild et al., 1931). The size of the nerve fibers changes from the base of the cochlea to its summit. According to Trevisi (1959), the volume of the nerve cell at the basal helix in the guinea pig is twice as high as at the apical helix.

The body of a bipolar nerve cell of the spiral ganglion has a swollen, spindle-shaped form and is covered from the surface with a capsule of satellites covering it. The dimensions of the neurons do not exceed 13-16 μ (Alexander, 1901). Wittmaack (1904) described the presence of a thin, pulpy membrane around the cytoplasm of the nerve cell under the satellite, the existence of which at the present time can be confirmed with the aid of an electron microscope (Engström and Wersäll, 1958c; Rosemblut and Palay, 1961; Rosemblut, 1962). The nerve cell contains a round large nucleus with one nucleolus located in its center. In the cytoplasm there is a large number of different organoids and mitochondria. Nissl's substance, which is abundantly represented, consists in the majority of cases of granulated endoplasmatic cisterns and RNA particles scattered over a great space. Between the cytoplasmic organelles, are found countless thin threads but their number greatly varies.

Rosemblut (1962) described two types of ganglionous cells, the spiral and vestibular ganglia of the rat. One type of ganglia cell is characterized by the presence of a large number of densely located clumps of Nissl's substance and contains few neurophils, another has few RNA granules but many neurophils. According to the data of Hyden (1943) an acoustic load imparts a number of essential devia-

tions in the quantitative relations of RNA, on the basis of which it is possible to judge the functional activity of neurons of the spiral ganglion.

In the cytoplasm of neurons Golgi's apparatus is found which, according to Kolmer's data (1927), is basically located in the regions of dendrite origin. Electron microscope investigations also note the unique vacuolized nature of the nucleolus. In the cytoplasm are contained vesicles provided with fissures or cavities (α -cistern), surrounded by membrane around 50 Å thick. On the surface of the membranes are granules with a diameter of around 100 Å, which are typical even for other neurons (Sjöstrand and Hanson, 1954; Palade and Sickevitz, 1956). Around these membranes there is another system of nongranulated γ -membranes primarily corresponding to Golgi's apparatus and a significant number of mitochondria with irregular internal membranes. Mitochondria are especially numerous in the region of neurite origin. Between the membranes in the mitochondria there is a significant number of small granules analogous to those which are located on the granulated membranes.

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Peripheral processes, i.e., dendrites of the ganglionic cells, are distinguished by their radial orientation and are covered with pulpy Schwann membranes. Their diameter is from 1-9 μ (Engström and Wersäll, 1958). They are directed toward the Lamina spiralis membranacea, located in its canals (Habenula perforata). Some of the fibers have a spiral direction; evidently, they are efferent and originate from the above mentioned Rasmussen's bundle (Fernandez, 1951; Lorente de No, 1937; Portmann and Portmann, 1954; Andrzejewski, 1955).

Dendrites, before leaving the Habenula perforata with the transition to the basilar membrane, lose their pulpy Schwann fibers and become pulpless. They turn somewhat into the region of internal hair cells. The other part is directed through the tunnel and Nuel's cavity and turn into the region of the external hair cells where their bases end in terminals. In addition, two complex spiral plexuses are formed along the entire cochlear canal. One of these is located in the region of the base of the internal hair cells and is called the internal spiral plexus of Corti's organ, the other in the region of the bases of the external hair cells and is called the external spiral plexus of Corti's organ (Boettcher, 1869; Retzius, 1884; Cajal, 1909; Held, 1926; Kolmer, 1927; Lorente de No, 1937; Poljak, 1946; Palumbi, 1950; Portmann, et al., 1953).

Despite the fact that Corti's organ of birds and mammals developed from one ancestral receptor portion the Papilla basilaris, in their structural organization there are a number of differences. Thus instead of two types of receptor cells, internal and external hair cells of Corti's organ in mammals, the hair cells of Corti's organ of birds are represented by only one type of short cylindrical elements, also provided with a cuticular lamina and crowned with sensory hairs on their apical surface. In mammals, the sensory

hairs are represented by a bundle of immobile stereocilia; instead of a kinocilium there is only a basal corpuscle in their apical portion. In birds, as was noted, this is a bundle of stereocilia which stands opposite one normally developed mobile kinocilium in a definite direction. Another essential difference is the fact that in birds the stereocilia are attached to the tectorial membrane which possesses a free and independent rhythm of motion in relation to hair cells with stimulation of the organs. However, the stereocilia of hair cells, in mammals and especially in birds, proved to be especially rich in specific ACE, which allows us to consider them as unique enzyme antennas connected with the cholinergic system and stimulated by means of mechanochemical processes. Significant differences must be noted in correspondence between receptor and supporting cells of birds and mammals. In mammals external hair cells are surrounded by endolymph; in birds they lie close to the supporting cells, just as the external hair cells of Corti's organ of mammals. In birds and mammals, the nature of innervation of receptor cells by means of light afferents and dark efferents of nerve endings which are characterized by high acetylcholinesterase activity, correspond. The same thing relates even to the very high activity of dehydrogenase located in the synaptic mitochondria in Corti's organ in birds and mammals. There are a number of characteristics differentiating the supporting cells of birds and mammals; for example, the absence of fibrillous structure in supporting cells in birds. In birds it has still not been possible to reveal morphological differences connected with the localization of perception of high and low frequencies which is so characteristic for receptor cells of Corti's organ in mammals, depending on their position on one helix of the cochlea or another.

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As a result of the comparison of Corti's organ of mammals and birds, it is possible to come to the conclusion that in the latter the subcellular organization of receptor cells in the connection with the tectorial membrane and also the general nature of interrelationship with the support elements is very similar to the subcellular organization of the receptor structures of the ampullar cristae and saccular and utricular maculae; i.e., the phylogenetically more ancient portions of the labyrinth, of which they are a derivative. Consequently, Corti's organ of birds (in comparison with Corti's organ of mammals) preserves a number of characteristics attesting to a certain primitiveness of its cellular and subcellular organization. However, this does not hinder the receptor cells of the avian Corti's organ to perceive practically the same frequency bands which are perceived by mammals.

The Function of Corti's Organ of Mammals

Several studies have been dedicated to the investigation of the function of Corti's organ of mammals. In a number of works Békésy (1948, 1949, 1958, 1960), with the aid of intravital observations, investigated in detail the entire mechanism of transmission of motions of the tympanic membrane arising through acoustic activities

transmitted through the auditory ossicles (malleus, incus and stapes) in the vestibular and then in the tympanic ladder, where they are damped in the region of the round window. The studies of Békésy were basically dedicated to the further conversion of these oscillations into motion of the cochlear canal and basilar membrane, which actively transmits auditory stimuli to the hair cells. Thus it was shown that a displacement of hydrostatic pressures of the peri- and endolymph occurred in the broadest portion of the basilar membrane, corresponding to the summit of the cochlear canal, 200 times greater than the displacement at the point removed by 33 mm from the stapes. Békésy (1949) discovered also that with an increase in frequency of vibration, the site of maximum reaction is transferred toward the stapes. Waves travelling along the cochlear canal, which arose on the action of auditory frequencies, were investigated (Békésy, 1947), which showed that the further the helices of the cochlea were situated from the oval window, the greater this motion was removed from the motion of the stapes. The motion of the cochlea inside the helix has the nature of a wave which is shortened and slowed according to its motion along the cochlea. In the region of the helicotrema it is very suppressed and arrives here 6 msec after the acoustic influence. On models of the cochlea, Békésy (1949) observed, in addition to a traveling wave, two other supplementary small vortices on each of the sides of the basilar membrane, appearing above the region of maximum amplitude of vibration. These vortices, according to the amount of frequency increase, are moved towards the narrower end of the basilar membrane, i.e., toward the base of the cochlea, and according to the amount of decrease in frequency towards its broader end, the summit. Thus at the moment of acoustic action, along the entire cochlea canal, traveling waves arise over almost the entire scale of audible frequencies. /56

On the basis of a large number of experiments it was shown that the cochlea receives low frequencies along the entire canal. High frequencies are perceived only in the region of the lower helix.

Thus it became known that the perception of sound by hair cells is connected with the motion of individual structures of the cochlea, chiefly the peri- and endolymph and the basilar and tectorial membranes. These motions lead to the excitation of receptor cells. However, the mechanism of primary reception itself remains unexplained at the present time.

Likewise the biopotentials arising with the perception of auditory waves by the organ of hearing are well studied in works of Davis (Davis, 1956a, 1956b, 1957, 1958; Davis et al., 1949; Davis et al., 1951; Davis et al., 1952). In the cochlea are found:

(1) Rest currents, negative at 50-80 mV in relation to the perilymph. Also the endolymph, i.e., positive potential, has rest currents. The dependency of rest currents upon intensity of aerobic exchange was established.

(2) Action currents which are generated by nerve fibers of the auditory nerve.

(3) Microphone effects representing alternating currents of the cochlea characterizing the response of receptor cells to acoustic action. The microphone effect consists of aerobe and anaerobe components. The latter is preserved after the death of the animal.

(4) Summation potential connected with electrical response to acoustic action on the part of the inner hair cells.

Békésy (1952) and Davis (1957) assumed that the summation potential and the microphone effect arise due to the bending of the stereocilia under the action of cutting forces elicited by the motion of the basilar and tectorial membrane. The bending of the stereocilia toward the surface makes the cochlear canal more negative; bending toward the center makes it more positive. Bending of the stereocilia along the spiral of Corti's organ does not have physiological effect. Critical directions of the stereocilia for internal hair cells are parallel to the axis of the cochlea. Moreover, in due time the idea was expressed that the stereocilia of receptor cells of Corti's organ possess the characteristics of piezocrystals (Guild, 1937) but those electrical potentials which could arise are insignificantly small in comparison with the amount of electrical energy of the cochlea. Intravital observations however, do not allow one to see the flexion of the stereocilia with any reliability (Vinnikov and Titova, 1961) which is an important fact testifying against the similar mechanism of excitation of receptor cells of the cochlea. In recent years, Engström, Ades and Hawkins, Jr. (1962) expressed the opinion that in Corti's organ it may not be a matter of flexion of the stereocilia, but that they act as levers which passively transmit cutting motion of the tectorial membrane to the cuticle of the receptor cell, and through it to the basal corpuscle and its accompanying organoids.

Functional Shifts in the Structural and Cytochemical Organization of Inner Ear Receptors

The above-given data on the cytochemical and histophysiological investigations of the vertebrate utricle and the mammalian Corti's organ showed that chemical, biologically active substances such as nucleic acids, proteins and functional groups of protein molecules, glycogen and a number of hydrolytic and respiratory enzymes, are not only structural components of receptor cells participating in the general metabolic processes, but also they simultaneously define their specific function. Data obtained on Corti's organ was later confirmed in a number of studies by foreign investigators (Vosteen, 1964; Beck, 1964; Rauch, 1964, and others).

Further investigations of the vestibular part of the labyrinth and, in particular, of the organ of gravitation using representatives of all classes of vertebrates revealed analogous regularities. Thus

it was shown that with the action of high G-forces on an animal by a radial acceleration of 10 g in the spine-to-chest direction, in the pitcher-shaped cells of the utricle (Type I cells) there takes place an extinction of cytoplasmic RNA located in the basal part of the cells in the form of a granular accumulation, general or total protein and functional groups of protein molecules. Simultaneously, in the nuclei of the cells a shift of the nucleolus toward the basal pole and a subsequent output of RNA of the nucleolus through the nuclear membrane into the cytoplasm of the cell is observed. Under the electron microscope it is possible to observe how such a shifting of the nucleolus toward the basal pole proceeds and is filtered through the well-known pores of the nuclear membrane composing its ribosome. In this portion of the cytoplasm of the receptor cells, simultaneously there is observed a process of formation of membranes of the endoplasmatic reticulum, evidently connected with the external nuclear membrane present in the shape of a conusoid spiral. Ribosomes passing from the nucleus also settle on these membranes. Under a light microscope this formation has the shape of a conusoid body, brightly stained with pironine and forming its own base toward the basal pole of the nucleus. These cytophotometric investigations allowed us to come to the conclusion that after the ribosomes of the nucleolus settle on the surface of the membranes of the endoplasmatic reticulum, a focus of local protein synthesis arises here, and the protein content in the cell is sharply elevated.

A comparison of histochemical data and changes in nucleolic and cytoplasmatic RNA with the data of cytophotometry allows one to draw the following conclusion: changes in optical density of protein as a result of single action (on the guinea pig) of an overload of radial acceleration show that sharp rises in the protein in the curves of optical density always follow an increase of newly formed granular accumulations of RNA in the cell. The size of the elevation of the curves drops sharply after vacuolization of the basal portions of the cell appears, and pycnosis of the nuclei (from the 5th day) arises. Results of the action by repeated rotation eliciting a secondary output of RNA from the nucleolus into the cytoplasm are various and depend upon the stage of protein synthesis which is proceeding when repeated action occurs. Repeated action has the most favorable consequences when it falls at the stage of formation of the accumulations of granular RNA in the cytoplasm with a minimum content of protein in the cytoplasm, i.e., when repeated action takes place after the beginning of the process of protein synthesis. Repeated action at maximum points of protein synthesis unavoidably leads to its destruction and to a sharp drop in protein content. These stages may be considered critical. The processes of multiple, repeated output of nucleolic RNA and the increase in protein synthesis lead to "hypertension" of the cell, which is expressed by the appearance of vacuolization, not only of the cytoplasm but also of the nuclei, and leads to pycnosis of several nuclei. This process abates on the 15-th day after the initial activity.

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Summarizing these data it is possible to say that the process

we studied in which nucleolic RNA leaves the nucleus under the action of an overload of radial acceleration on the utricle is inseparably connected with the synthesis of cytoplasmic RNA, due to which even protein synthesis is strengthened. This process evidently is physiological for the cell, and the action of an overload of radial acceleration upon the animal only stimulates this process and makes it more synchronized. The stimulation or, inversely, suppression of protein synthesis with repeated rotation of the animal, depending upon the stage of formation of cytoplasmic RNA and synthesis of protein, upon which the repeated action falls, indicates that the output of nucleolic RNA stimulates not only the synthesis of cytoplasmic RNA, but also protein synthesis (Vinnikov, Gazenko, et al., 1963, 1965, 1966).

With histochemical investigations of Corti's organ in mammals it was established that, as opposed to neurons of the spinal ganglion, hair cells of Corti's organ significantly more demonstratively react to an elevated functional G-force. This reaction of excitation is characterized by a change in the shape of the cell body, dimensions of the nucleus, and distributions of RNA and DNA concentration. A shift of DNA to the periphery of increasing nuclei and a lowering of the concentration of RNA in the cytoplasm of such a cell attests to the strengthened processes of segmentation of proteins connected with RNA, intensively dispersing during excitation, when protein synthesis does not succeed in compensating for its disintegration. A decrease in the sizes or the contraction of the nuclei of individual hair cells and the enrichment of their nucleus and cytoplasm with diffuse RNA attests to the fact that such elements begin to synthesize proteins connected with RNA again and, additionally, are returning from the excited state to the original condition of relative rest. In this period, resynthesis of protein predominates over disintegration of protein (Vinnikov and Titova, 1961).

This information manifestly shows the definite participation of DNA and RNA in the processes of excitation of hair cells of Corti's organ and the utricle. The clear pattern of an increase in size of the nucleus which is observed in Corti's organ and the marginal position of DNA in receptor cells, which is accompanied by a lowering of the concentration of RNA in the cytoplasm, evidently gives evidence of processes of depolymerization of the latter and of disintegration of the proteins connected with it. On the other hand, the contraction of the nucleus and the accumulation of RNA and DNA in it, and also the strengthening of the concentration of RNA in the cytoplasm of such hair cells, indicates a strengthening in the process of resynthesis of nucleic acids and proteins connected with them. Such hair cells with contracted nuclei and with elevated concentrations of nucleic acids are often found after long periods of acoustic action, and allow one to conclude that in individual receptor cells restorative processes begin during advanced stages of excitation.

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It is necessary to note that in Corti's organ the internal hair cells appear to be more stable. Changes in nuclear sizes in them and the distribution of DNA and RNA are less distinct in comparison with the external hair cells. This once more emphasizes the great sensitivity of external hair cells to adequate acoustic activity. The change in the distribution of nucleic acids in Corti's organ is strictly subordinated to the general physiological gradient of the cochlea. With high frequency activity it changes in the receptors located on the lower helices with activities of low frequencies on the upper helices.

The change in total protein and functional groups of protein molecules is especially demonstrative in receptor cells of the utricular macula and Corti's organ. Studying these changes gives us some idea of the protein exchange in the receptors of the inner ear and the conditions both of relative rest and of adequate G-force. As the investigations which were conducted indicated, the total protein and functional groups of protein molecules in a greater or smaller concentration become part of all receptor structures of the inner ear and neurons of the vestibular and spiral ganglia. The changes in their location and concentration with adequate G-force indicates that in an excited cell the question is one of a general process connected with all the protein structures in their morphological, biochemical and biophysical expressions. This process is the result of the interaction of the protein component not only in the substrata and enzymes, but even with the nucleic acids responsible for protein synthesis. At the same time it is characterized by reversible denaturation of the protein component. Evidently, elevation of the protein concentration and its functional groups in the cytoplasm and in the nucleus of receptor cells, and also in the neurons of the spiral ganglion, after a 15-minute activity, and depression of these after 1, 2, 4 and 6 hour action, is connected with this process.

According to biochemical data (Ungar, 1957), an elevation in the amount of SH- and COOH-groups of protein corresponds to the condition of excitation of the cells and is connected with the denaturation of their protein molecules, whereby an opening of the polypeptide change takes place. A decrease in the concentration of protein after prolonged activity, according to biochemical data (Hyden, 1955), connected with disintegration of proteins, i.e., with an increase in protein exchange, is largely affected by proteolytic enzymes which denaturize protein molecules before breaking the peptide bonds (Lindeström-Lang, 1952). Products of protein hydrolysis during fixation are washed out of the cells, a process which is histochemically expressed in the drop in concentration of total protein and functional groups of protein molecules.

The fact of a high glycogen concentration in the external hair cells and its change during functioning indicates the large role of anaerobic exchange in the life activity of Corti's organ. The histochemically established fact of a drop in the concentration of

glycogen in excited hair cells is connected evidently to the change in the ratio of the glycogen fractions. In excited hair cells of Corti's organ there is a sharp increase in the amount of glycogen directly connected with protein, which is manifested only by biochemical means (Leibson et al., 1961). It is possible that this reflects even the general denatured shifts of the protein substratum of receptor cells.

The investigation of specific enzyme of neural conduction, i.e., hydrolyzing acetylcholine-acetylcholinesterase, showed its location in the region of the synapsis of all inner ear receptor elements in all vertebrates and a change in its enzymatic activity and location with specific activity on the utricle or on the mammalian organ of hearing.

Results obtained with the comparison of biochemical and electrophysiological data allow one to come to the conclusion that during the conduction of a nerve impulse in the region of the synapsis, both in the utricular macula and in the receptor epithelium of the cristae of the semicircular canals, and in the appearance and conduction of a nerve impulse in the region of the intraorganic synapses of the cochlea, the cholinergic system plays a fundamental role which is connected with the mechanism of acetylcholine action and, in particular, with its hydrolysis by a specific enzyme, i.e., acetylcholinesterase. Adequate specific G-force first provides an elevation in the activity of the enzyme, but gradually the loss of acetylcholine and its depletion finally leads to a drop in the activity of hydrolyzing enzymes.

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Histochemical investigations of a number of dehydrogenases in the utricle of several vertebrate animals established their very high enzyme activity in the region of the so-called scyphoid synapses of Type I cells which increases and then regularly lowers under the action of high G-forces from radial acceleration. Further investigations of the changes in the region of the synapses of utricular receptor cells in a number of vertebrate animals, made with the aid of an electron microscope, show that in the synaptic region of the utricular macula, after high G-force action of radial acceleration on the animal, in addition to a change in the enzyme activity in a number of respiratory enzymes located in the mitochondria, there is also a spatial shift of the mitochondria and of the phenomenon of their application to the presynaptic membrane of the receptor cell and efferent nerve ending. Besides the phenomenon of application of mitochondria to the presynaptic membrane, the mitochondria also swell, which is especially pronounced in the presynaptic mitochondria both of the bud-shaped and of the scyphoid nerve endings.

Histochemical investigations of changes in enzyme activity of the succinate dehydrogenase and receptor cells of the mammalian Corti's organ, of the nerve endings leading to them and of the neurons of the spiral ganglion, demonstrate the presence of similar effects

of action upon Corti's organ by sound of a definite frequency. In this regard, the especially clear results of the elevation in SDH activity for short periods of acoustic action and the drop in SDH activity for extended periods are manifested by the mitochondria of external hair cells. The mitochondria prove to be the structures of the hair cells which during excitation are redistributed in the cytoplasm and simultaneously swell. The drop in enzyme activity noted during the later stages of acoustic action and the depletion of enzyme activity reflect conditions of inhibition of the hair cells.

Results obtained with the aid of an electron microscope are in good agreement with data of histochemical investigations; i.e., they show an elevation and then a drop of dehydrogenase in the synaptic mitochondria of the utricle in Corti's organ during their specific stimulation. The attachment of mitochondria to the presynaptic membrane well illustrates the energetic participation of mitochondria in the conduction of the nerve impulse in the region of synapsis. This attachment of mitochondria simultaneously attests to the direct participation of the Krebs cycle, oxidized phosphorylation and electron transfer (Green and Hatefi, 1961) in the conduction of a nerve impulse.

Consequently, neural conduction through a synapse is accomplished with the aid of not only a specific cholinergic mechanism but also a general, nonspecific, energetic mechanism connected with the function of the mitochondria; the excited condition of a receptor cell is connected with general processes of chemical conversion of nucleic acids and proteins as well as of the enzymes in their substrata.

Data on the Comparative Morphology of the Inner Ear of Vertebrates and its Receptor Structures

The labyrinth of vertebrates arose in phylogenesis on the basis of the lateral line organs; as a result, today these organs are united into a single acoustico-lateral system, at the base of the receptor formations of which lies the secondarily sensitive receptor cell (Vinnikov, 1946). Secondarily sensitive cells appeared at the dawn of vertebrate evolution. As far as it is possible to judge, according to sufficiently reliable paleontological data (Bystrov, 1957), in primitive agnathous vertebrates (Agnatha), which in the Silurian era were already adapted to conditions of life in fresh water basins, the system of lateral line organs was well developed. This system has been preserved up to the present time in fish and aquatic amphibians in which the secondarily sensitive cells are receptor elements. This system in the agnathous vertebrates consisted of tubules or canals which were located in the aspidine substance of their shells which have been preserved up to our time. The tubules opened on the exterior surface of the armor. Thus, this entire system of lateral line organs communicated with the external aqueous medium, the vibrations of which appeared to be specific stimulation for it, if we can judge by the presently extant representatives of

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fish and amphibians. In presently existing fish, the canals of the lateral line organs are filled with a mucilaginous substance and communicate with the external medium, either directly with the aid of pores or, as in several types, indirectly, since these pores are covered with a membrane. In fish, as in aquatic amphibians, the receptor elements of lateral line organs may be located on the surface of the skin. According to the data of N.I. Disler (1960), the structure and development of lateral line organs reflects the ecology of the various types of fishes. In several amphibians, the lateral line organs are subject to rhythmic seasonal changes. According to the data of G.A. Malyukina (1955), the lateral line organs of fishes, along with perceiving oscillations of the water, evidently also perceived the entire band of infrasonic frequencies, and the lowest auditory frequencies up to 25 Hz. Dijkgraff (1954) denies that the lateral line organs are capable of perceiving auditory frequencies.

On the basis of the system of the lateral line organs, the labyrinth, originally communicating with the external environment, develops; i.e., the inner ear, which we find in the Silurian in representatives of agnathous fishes such as the Osteostraci. According to the data of Stensiö (1927), in a representative of the Osteostraci-Kiaeraspus such a labyrinth had only two semicircular canals: an anterior and a posterior one. The lateral canal was still absent in these. Of contemporary forms, such a characteristic is found in the labyrinth of the cyclostomate (Tret'yakov, 1916; de Burlet, 1934). The question as to whether the simplified structure of the cyclostomate labyrinth is an original or a secondary result of a parasitic form of life has not been decisively answered to date. It is possible to say with any assurance only that the organ at the very beginning of the labyrinth constructed symmetrically and divided into an anterior and posterior half. This division may be traced in an entire series of vertebrates, which gives us the possibility in defining homologous formations, even in those cases when the original symmetry has been completely destroyed (de Burlet, 1934). The anterior half of the labyrinth in all vertebrates is innervated by the Ramus anterior; the posterior is innervated by the Ramus posterior of the eighth nerve.

The labyrinth of the hagfish possesses two ampullae. In addition, the two semicircular canals turn directly into one another. Each ampulla, respectively, possesses its own receptor formation, i.e., a terminal organ.

In the lamprey (Petramyzon) there are two independent, semicircular canals, and (especially characteristic for the labyrinth of the lamprey) two spaces or chambers, lined with fibrillar epithelium, which are formed from the walls of the utriculus and the lower section of the crus communa. The fibrillar epithelial cells of the chambers are provided with long mobile flagelli, which bring the fluid inside the labyrinth into motion. All of the functions of these formations remain a mystery at the present time, insofar as

motion of the fluid is limited by the space of the chambers, and does not encompass the semicircular canals and other parts of the labyrinth where the fluid remains immobile. The small, elongated utricle is located between the ampullae. At its base two projections are separated, one behind the other; the anterior is smaller, evidently corresponding to the sacculus; the posterior is larger, and is usually designated as the lagena (de Burlet, 1934).

The labyrinth of selachians is distinguished by the great autonomy of semicircular canals which are completely separated from the remaining portions of the auditory vesicle by short connecting tubules. Here, moreover, there is a completely formed, third semicircular canal: the horizontal one. It destroys the symmetry which is present in the labyrinth of cyclostomate; however, the rest of the organs are completely similar. It is important that this third semicircular canal is related only to the anterior vertical canal, since its anterior end and the ampulla are only open in this canal.

The demarcation of the labyrinth into anterior and posterior halves decreases in significance, and gradually a single apparatus of semicircular canals is formed, a good example of which is the labyrinth of the chimaera which is very close to the labyrinth of the dipnoi. In this labyrinth the maculae lie in a vertically located open sac which is divided into an anterior and posterior section by means of a fissure. The anterior is designated the recessus utriculi and contains the macula utriculi; the posterior is the united macula sacculi and macula lagenae. The general opening is called the otosacculus with a section of the semicircular canals. The ductus endolymphaticus opens near it. It is possible to assume that a similar labyrinth corresponds to the division of the labyrinth on the pars superior and the pars inferior (de Burlet, 1934). The difference between this labyrinth and the labyrinth of the higher vertebrates consists of the fact that in the latter the utricular macula is located always together with the apparatus of the semicircular canal. That part of the labyrinth which, in the higher vertebrates, is designated the utricle, is always distinguished by the presence of the utricular macula. The upper part of the labyrinth of the chimaera is only a complex of semicircular canals. That which is designated here as the utricle is a longitudinally elongated sleeve, in which the 5 ends of the semicircular canals open and which contains the utricular macula consisting of a section of the semicircular canals and of part of the otosacculus, just as that which contains the macula utriculi (recessus utriculi). In numerous forms, this recessus utriculi preserves a certain independence; for example, it possesses a fissure which does not completely separate it from the remaining utricular portions. The remnant of the otosacculus is designated the sacculus. The latter is situated on the lower circumference of the labyrinth. It is distinguished by its great variability in size and shape, and also by its ability to form new sections. On the other hand, the utricle with the apparatus of semicircular canals, is a more conservative part of the labyrinth (corresponding to the pars superior labyrinthi), which preserves the principle of

immutability. The lower end of the crus communi can be continued and designated the sinus superior utriculi; if the posterior end of the utriculus into which the ampulla of the posterior vertical canal falls is extended, then it is designated the sinus posterior utriculi. However, all these variations of the utriculus do not have great significance (de Burlet, 1934). The macula neglecta, which is located in the utriculus in bony fishes and was first discovered by Retzius, is considered by de Burlet as a derivative crista. Its covering formation is of the nature of a cupula, which provides the grounds for naming it the crista quarta. However, several fishes have an otolith membrane rather than a cupula. These differences led de Burlet to propose a neutral designation, the papilla neglecta, which, however, is not acknowledged in contemporary scientific literature.

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The macula neglecta is present in the inner ear and representative of all classes of vertebrates, and it is most often found and most highly developed in the fishes. In amphibians, it is often absent. In reptiles and in birds it varies greatly, both in structure and in location, and it is absent in many mammals. It is either located at the base of the utriculus or is moved forward along its lateral wall, between the opening of the utricular-saccular canal and the posterior ampulla. Its function is unknown, but in the opinion of de Burlet, it cannot be similar to the function of the other cristae, insofar as it is located above and below in the endolymph (Ayers, 1891; de Burlet, 1934).

The differences which are observed in the pars inferior labyrinthi in various known classes of vertebrates; i.e., those which are connected with the evolutionary advances of the sacculus, are of more essential and progressive significance. They relate both to the morphological and to the functional change in the nature of the organ. Thus, the macula lagenae and the new organ, the papilla basilaris, which arises in the higher vertebrates, are connected with the system of the sacculus.

As opposed to the saccular and utricular maculae, the lagenar macula is absent in several vertebrates. Thus, among the mammals, it is only found in the monotremata, and is absent in all other forms. Even in the fishes, there are individual forms in which the lagenar macula is absent. In others, it lies in a more or less unique process of the sacculus, and its spatial orientation corresponds to the saccular macula. This sign is true in the amphibians and changes only in the Sauropsida, where it is located together with a papilla basilaris. At the same time, as a development of this acoustic formation, the lagenar macula occupies a still more peripheral position, and in birds, it finally is located at the end of the cochlear canal in the extended recessus lagenaris. For the lagena macula of birds, it is characteristic that it lacks a fundamental plane, and its surface, on the whole, appears to be bent, even in the form of a spiral. How it is spatially oriented has not been established in past investigations, and still remains unknown.

De Burlet relates the complete and sudden disappearance of the lagenar macula in mammals to the shortening of the cochlea, since, with the appearance of helices, the end of the cochlear canal loses its firm spatial orientation.

The function of the lagenar spot has remained unexplainable up to the present time. Several authors connect it with the flight capabilities (de Burlet, 1934); others proceed from the characteristics of its innervation in birds and relate it to hearing through bone conductivity (Pumphrey, 1961). However, due to the presence of this sensory spot in all lower vertebrates, it is difficult to imagine that its function is limited only to these tasks.

The papilla basilaris in the Sauropsida and mammals is developed in a new section of the labyrinth, the organ of hearing (papilla acustica basilaris of the higher reptiles and birds and Corti's organ of mammals). It is located in a blindly closed cochlear canal, which in birds makes a half turn, and preserves a connection with the lagena at its end; in the mammals it is greatly increased in length and forms a spirally shortened cochlea which loses its original connection with the lagena, preserving this only in the monotremate mammals. It is interesting that despite the movement of vertebrates onto the dry land, their labyrinth and cochlea, as before, remains immersed in the internal medium of the organism. In addition, it seems to preserve, in the constitution of its open space, the same liquid medium (endolymph and perilymph), the wave oscillations of which, in due course, occasion stimulation of the receptor structures of the lateral line. Thus, one more change in function took place, and from the organ of equilibrium a new morphophysiological formation was separated: the organ of hearing. Despite the complex evolution of the lateral line organs, on the basis of its new derivative, the organ of balance and hearing, the original receptor form is preserved as before; i.e., secondarily sensitive receptor cells. Thus, the lateral line organs, just as the other sense organs, not only retained their significance with the transition from an aquatic medium to a terrestrial or aerial one, but furthermore they acquired still greater significance, attained a greater structural complexity and an even greater perfection in the higher vertebrates, especially in the mammals (Shmal'hausen, 1938, 1940). In the lateral line, their stimulation was accomplished directly by vibration of the fluid current or indirectly by vibration of their gelatinous structures, which supplementarily developed on the surface of the receptor cells. These same gelatinous structures, in the form of cupulas, developed also on the surface of the hair cells of the auditory crests in the ampullae of the semicircular canals. The motion of endolymph, in connection with the change of the body position, leads to a deflection of the cupulae, thanks to which, with the aid of the hairs, the hair cells of the cristae are stimulated and excited. In the hair cells of the sacculus and utricle, the process is more complicated. On the surface of the adjacent otolith membrane, heavy crystals containing calcium salts, i.e. otoliths or otoconia, are developed. Their pressure constantly stimu-

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lates the secondary sensory cells of the auditory spots, and their excitation constantly signals the body position in relation to the center of the terrestrial gravitation. The slipping of otoliths during inclination of the head or trunk strengthens or lowers this stimulation. In Corti's organ, stimulation of the hair cells is also connected with the wave-like motions of the basilar and tectorial membranes, and with the strictly regulated wave-like motions of the endolymph.

However, the recent electron microscope, histochemical and electrophysiological investigations (Flock and Wersäll, 1962a, 1962b; Flock, 1964; Löwenstein et al., 1964) showed that the principle of stimulation of the hair cells of the labyrinth is connected with polarization of its mobile kinocilium. However, the principle of primary receptor elements in the hair cells of the labyrinth remains unexplained at the present time.

In addition, it is necessary to note that the labyrinth's numerous functions are not so completely delimited from each other as was thought earlier. It is assumed that only the auditory spot of the utricle can be considered a truly static receptor perceiving the position of the animal in the gravitational field. The sacculus simultaneously fulfills other functions; for example, the perception of vibrations, and even acoustic stimulation. The same relates to the receptors of the lateral line (Buddenbrock, 1952; Malyukina, 1955). For example, in bony fishes the removal or destruction of the saccular otolith does not influence reflexes connected with equilibrium, while the destruction of the utricle leads to the same results as the destruction of the whole labyrinth (Maxwell and Huddelston, 1923; von Frish, 1929; Werner, 1929). From amphibians, similar data were obtained (Huddelston, 1928; McNally and Tait, 1925, 1934, 1935). Analogous results were obtained on birds (Benjamins and Huizinga, 1926, 1927, 1928) and on mammals (Versteegh, 1927). /65

In a number of studies, Löwenstein and colleagues (Löwenstein and Sand, 1940; Löwenstein and Roberts, 1948; Löwenstein, 1953); with the aid of shunt conduction of action potentials from the labyrinth of the skate, discovered statoreceptors in all 3 portions of the labyrinth provided with otolith. In addition, they showed that the utricle is completely specialized as a statoreceptor. The sacculus is a statoreceptor in its posterior part, while the rest of its surface responds to vibrational stimulation. The lagena is an antagonist in relation to the utricle and sacculus. However, in the pigeon, the sacculus and lagena can also fulfill the role of an organ of equilibrium (Huizinga and Benjamins, 1929).

The third static organ; i.e., the lagena, was physiologically studied only in the frogs and skates. McNaughton and McNally (1946) discovered that if both lagenae remain in the frog, and the rest of the labyrinth is removed, then it preserves equilibrium better than a frog with a completely removed labyrinth. If the nerve going to the lagena on one side is severed, then the frog holds its head

higher on the operated side. Evidently, the lagena maintains the tone of certain muscles. The inter-relationships between receptors of the utricle, saccule and lagena are unclear at the present time. English authors assume that the question is one of their antagonisms. According to von Frisch (1929), several fishes are capable of hearing with the aid of the lagena. Evidently, the question is one of mixed receptor capacities of this portion of the labyrinth (Vinnikov and Titova, 1961).

As far as the auditory crests are concerned, and according to the generally accepted concept, they can perceive stimulations caused by acceleration or retardation of motion (change in angular acceleration).

Thus, at the basis of all receptor structures of the labyrinth, including Corti's organ, lie the hair cells. The light microscope showed that they are present in all vertebrates with a more or less cylindrical shape. The electron micron revealed the presence of a superficial cuticle, an endoplasmatic network, mitochondria, etc. (Wersäll, 1956). The hair cells are located on the supporting cells, and are differentiated by the unusually strong synaptic system formed by the nerve fibers which begin from the bipolar vestibular neurons. Simultaneously, in recent years the presence of efferent innervation was discovered in them (Rasmussen, 1946). Receptor cells bear very strong hairs on their summit, which, in the region of the auditory spots, support the otolith membrane with its heavy otolith. In the region of the crests in the semicircular canal, these hairs usually attain great sizes and pass inside the special canals of the cupula, made of a special gelatinous substance.

However, besides these basic general features of the structure of all receptor elements of the vertebrate labyrinth there are significant, still little explained, differences in their fine structure, and possibly even in the trigger mechanism of excitation. Thus, while in the labyrinth of the lower vertebrates there is one type of receptor hair cells in a receptor formation of the labyrinth of the higher vertebrates, Type I and Type II cells are clearly distinguished, and differ not only in the shape of the cellular body, but also in the presence and distribution of cytoplasmic organoids, and also by the means of innervation and the structure of nerve endings formed on them (Wersäll, 1954). It is necessary to note that the one type of receptor element of the lower vertebrate cannot be completely ranked with either one of the two receptor elements of the higher vertebrate since it bears similar features to both types (Engström, 1960a, 1960b; Löwenstein et al., 1964). Still greater differences may be found in the ultra-fine structure of the receptor elements of Corti's organ (the organ of hearing) in mammals. Here, there are also two types of receptor elements (i.e., internal and external receptor cells); the first preserves some features of similarity of ultra-fine structure with the vestibular receptor Type I cells, and the second evidently has gone further along the evolutionary path, and there are several features of structure in them (for example, an

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increase in membranes), which distinguish them from all the rest of the receptor elements of the inner ear of vertebrates. It is necessary to note that the basic role in the appearance of the fine differences between the receptor elements of various receptor formations of the labyrinth of vertebrates are played by the above-mentioned sensory hairs, and also the basal corpuscle and cuticular lamina connected with them. This is especially apparent in comparison with the characteristics of the structure of the external hair cells on various helices of the cochlea (Engström, Ades and Hawkins Jr., 1962). On all receptor cells of the labyrinth of vertebrates, excepting the internal and external hair cells of Corti's organ of mammals, besides the above-mentioned immobile sensory hairs (i.e., stereocilia) there is still one mobile hair, the kinocilium, having a characteristic fibrillar structure of 9 pair of peripheral, and two central fibrils. At the same time, the stereocilia are always connected with the cuticular lamina and pass into it with their root thread; the kinocilium is always located on the same portion of the cellular surface which remains free of cuticle and its fibrils continue inside the cytoplasm into the basal corpuscle located here. This kinocilium is absent only in the receptor cells of Corti's organ in mammals, but its basal corpuscle is still preserved (Engström, Ades and Hawkins Jr., 1962).

According to the most recent electron microscopic data, the presence of a kinocilium in receptor cells, in front or in back of the bundle of stereocilia, which are most often distributed in the form of a letter W, creates morphological and evidently physiological polarization of the cell. Investigations of Löwenstein and colleagues (Löwenstein et al., 1964) of the labyrinth of the skate showed that individual receptor formations of this labyrinth are distinguished chiefly by the distribution of kinocilia in the receptor organ. The absence of kinocilia in the auditory cells of Corti's organ of mammals indicates that undoubtedly not only a change in function took place here, but also in the process of evolution; even the principle of the trigger mechanism of excitation of the hair cells of the mammalian organ of hearing changed. Even more interesting is the fact that, according to the latest electron microscope data (Vinnikov, Osipova, Titova, Govardovskiy, 1965), in the organ of hearing of birds, the mobile kinocilium is preserved. It is necessary to note that the preservation of the basal corpuscle in all receptor cells, without exception, leads one to the thought that it evidently is one of the basic components of the mechanism of primary excitation of the hair cell. A change in the length of stereocilia on various helices on the cochlea of mammals attests to the fact that it has a certain, still unknown, significance in the process of perceiving various acoustic frequencies. The significance of the distribution of the stereocilia of internal hair cells in two parallel rows still remains a mystery. But it is difficult to judge this while the function of the internal hair cells still remains unexplained. The unequal size of stereocilia inside the bundle, with their increase in the direction toward the kinocilium, appears to be a general rule for all receptor cells, with the exception of the

internal hair cells of Corti's organ, where this question has not been investigated to date .

Thus, within the bounds of the labyrinth, the significant differences in the structure and function of hair cells have been successfully revealed, which attests to the fact that the evolutionary change in the functions of various receptor portions of the labyrinth was accompanied by a complicated complex of morphological and physiological changes. On the basis of the labyrinth, it is possible to be easily convinced that its receptor elements perform not one, but several functions. Of these, the function of maintaining equilibrium appears to be originally the primary one, and the auditory function seems secondary (Dohrn, 1875). Then, the auditory function is developed increasingly more intensively and leads to the appearance of a special organ of hearing, Corti's organ, in birds and mammals. However, in addition the original function is in no way weakened and plays its former role. Thus, the question is one of expansion of the former original function (Plate, 1925).

It is also necessary not to lose sight of the parallel evolutionary transformations which the branchial cleft and its bones entering the composition of the middle ear endure at this time. The appearance of openings covered by membranes by means of which the cavity of the labyrinth communicates with the external environment has great significance; we have in mind the round and oval windows and the mechanism of moving the auditory ossicles (malleus, incus and stapes) and the muscle connected with them (stapedius), which in turn are excited by oscillation of the tympanic membrane under the influence of air waves. With absolute "resonating" precision, they transmit acoustic waves to the endolymph and perilymph, filling the cavity of the cochlea, for example in mammals. Along the frequency of the oscillations of the membrane, the motion of the fluid and the change in its chemical composition are specific stimuli for the hair cells of Corti's organ (Vinnikov and Titova, 1961).

EMBRYONAL DEVELOPMENT OF THE INNER EAR

The development of the inner ear of vertebrates was investigated mainly at the beginning of the present century, using histolytic as well as some neurohistolytic and embryological methods, with the aid of optical microscopy. For the most part, these data do not provide a detailed description of the cytological differentiation of the receptor formations and are completely unsatisfactory in many instances. Studies of the embryogenesis of the ear at the level of electron microscopy are completely lacking in the literature available to us. Of equal rarity are reports on the cytochemistry of developing structures, not limited to the ear alone. In this regard, we should note the extremely interesting studies conducted by Levinson et al., on the histogenesis of the moto- and sensory neurons of the spinal cord in the embryos of chicks, rats and white mice and the cells of the auditory ganglion of the axolotl. Levinson (1961) mentions that the chemical maturation of neurons is accompanied by changes in the concentration of various substances, including RNA, protein, certain amino acids, enzymes, the ability of the neuron to store glycogen in its cytoplasm, etc. In Levinson's view, the process of development of the neurons is accompanied not only by a change in concentration, but also by a redistribution of various substances among the cell structures, i.e., their functionally conditioned cytoarchitectural changes. /68

In the present survey, we shall attempt to discuss the embryonal development of the ear of vertebrates on the basis of the data in the literature at our disposal, considering it from the standpoint of function whenever possible.

Morphological Studies of the Development of the Vestibular Apparatus

The early stages of development of the membranous labyrinth are similar in all vertebrates, and it is only at later stages that a number of characteristic features appear in representatives of the various classes of vertebrates. The first double rudiment of the labyrinth appears in the form of a thickening of the ectoderm, located on both sides of the still open neural lamina in the area of the future epencephalon (Bartelmez, 1922; Wilson, 1914; Ingalls, 1920). In human embryos, this occurs at the stage of 2-3 somites (O'Rahilly, 1963). At the 8 somite stage, these rudiments have become so distinct that they are already referred to as auditory placodes (Kupfer, 1891; Mitrofanov, 1892; Kappers, 1941). Huschke (1831) was the first to show that the membranous labyrinth develops from the superficial ectoderm (Streeter, 1906). Since those times, the number of papers dealing with studies of the auditory vesicle has grown considerably (Altmann, 1950); in 1939, Groth showed that the placodes in mammals develop from lateral folding of the neural lamina, leading to the formation of the ganglionic lamina. Near the auditory placodes and somewhat anterior to them is the rudimentary /69

acoustic-facial ganglion. Batten (1958) has shown that the auditory ganglion in the developing membranous labyrinth of the sheep develops from placodal cells that separate from the wall of the auditory vesicle. This was also demonstrated by van Campenhaut for the guinea pig (1935) and Theiler (1949) for man; the latter showed that the migration of cells from the wall of the auditory vesicle begins only after its closure and separation from the ectoderm. In the embryos of herring and salmon, the auditory placodes are connected to the rudimentary lateral line. This is indicative of a relationship between the two sensory organs, which have much in common in the adult animal. At the 14-15 somite stage of the human embryo, the center of the placode is embedded in the subjacent mesenchyma, forming a depression--the auditory fossa. In his study of the inner ear of the rabbit, Groth (1939) speaks of a lateral fissure, a zone of the labyrinth from which the auditory lamina develops. The auditory fossa gradually deepens and turns into the auditory vesicle. For a short time, it continues to be in communication with the gradually shrinking opening; at the 3 mm stage, this opening usually closes, but the connection between the vesicle and the superficial ectoderm is retained for a time by means of a strand of epithelium. At the 4 mm stage, this link is severed completely (Anson and Black, 1934). Only in the herring does this opening remain open during the entire lifetime of the animal; it becomes the ductus endolymphaticus which allows sea water to enter the labyrinth. In representatives of all other classes of vertebrates, the auditory vesicle is closed off completely from the ectoderm, and the question of whether the endolymphatic duct in these animals is homologous to that of the herring remains open. In any event, the endolymphatic duct and the vesicle are formed very early. In birds, they appear when the latter is still connected to the ectoderm and (as in other vertebrates) are formed from the medial wall of the auditory vesicle. In some fish (Dipnoi), amphibians (Axolotl, Anuren) and reptiles, the labyrinth is not formed by closing off the auditory vesicle, but by the formation of an epithelial induration originating in the deep layers of the epithelium. In this case, however, the sacculus and the ductus endolymphaticus are formed from the medial wall in embryos 6.3-6.7 mm long (Brunner, 1934). In man, the endolymphatic diverticulum is formed from the medial wall of the auditory vesicle. The endolymphatic vesicle in the 12 mm embryo becomes the endolymphatic canal and vesicle.

In further stages of development, the vesicle lengthens dorso-ventrally. The increase in size is the result of multiplication of the cells in the wall of the auditory vesicle, as well as an increase in the volume of the liquid with which it is filled, which in turn causes the walls to stretch (Ranzi, 1924; O'Rahilly, 1963). The division of the auditory vesicle into two smaller sacs is accompanied by the development of a lateral fold, which appears opposite the point where the ductus endolymphaticus empties. The lower of the two small sacs, called the pars inferior, gives rise to the sacculus and its derivative (i.e., the lagena and auditory papilla), and (in higher vertebrates) the cochlear canal as well; the upper one,

called the pars superior, becomes the utriculus and the semicircular canals. This division is characteristic of all vertebrates except the Cyclostomata; in certain teleostean fishes, the process continues until these two parts are completely separate (Bierbaum, 1914). The formation of the semicircular canals occurs in human embryos at stages 16, 17 and 18 (corresponding to lengths of 7-17 mm; cf. O'Rahilly, 1963). The separate parts of the wall of the upper section thicken and form protrusions in the shape of flat pockets at these locations. The opposite walls adjoin one another, the sites of adhesion are absorbed into one another, and the semicircular canals are formed from the peripheral parts of the pockets (Rathke, 1832; Böttcher, 1869; Bast et al., 1947; Kolmer, 1927; de Burlet, 1934; O'Rahilly, 1963). Usually, both vertical semicircular canals are formed from a common rudimentary structure and their posterior ends connect with the middle portion of the utriculus via the joint crus communaе or sinus superior utriculi. The other (anterior) ends of these semicircular canals connect directly with the utriculus, forming chambers (ampullae). The flat, pocket-shaped protrusion from which the horizontal (lateral) semicircular canal is formed usually closes somewhat later. The final development of this semicircular canal and its ampulla also usually lags behind that of the two vertical semicircular canals. At the same time that this process of resorption is taking place, the penetration of the mesenchyma is also occurring, and (in the opinion of de Burlet) the formation of the semicircular canals takes place under the influence of two factors: the active protrusion of the wall of the labyrinth and the inward growth of the mesenchyma. The relationship between these processes varies in different animals (de Burlet, 1934). The link between the utriculus and the sacculus can be formed in different ways. In mammals, it takes the form of a narrow channel, the ductus utriculosaccularis, into which the ductus endolymphaticus also empties (Anson and Black, 1934). According to Brunner (1934), the utriculus in human embryos at the 84 mm stage connects with the sacculus via the sacculo-endolymphatic canal; each is a branch of the endolymphatic canal, which assumes a V-shape. Later, a medial-dorsal fold is formed between the sacculo-endolymphatic canal and the sacculus. In 183 mm embryos, it becomes a special valve, the *volvula utriculiendolymphatica*, first described by Bast (1930). In other forms, the opening also remains narrow, and (in exceptional instances) the utriculus and sacculus are scarcely evident. There are also extreme cases when (as we saw earlier) the utriculus and sacculus are completely separate (Bierbaum, 1914). /70

As we have already seen, the sacculus is formed from the lower section; it can develop further protrusions to form the lagena, cochlea, etc. In some fishes, the bilateral fusion of this section forms the *canalis communicans transversus*, linking the two labyrinths.

It is approximately at this time that there is a considerable change in the positions of the utricular and saccular portions of the auditory vesicle as a result of their shifting. The utriculus rotates so that the endolymphatic duct and follicle occupy a medial

position; the sacculus, on the other hand, moving along the lengthwise axis of the auditory vesicle, shifts from a vertical to a more horizontal position. As a result, the semicircular canals gradually move laterally; the cochlea, developing in higher vertebrates, assumes a more medial position (Bast and Anson, 1949).

The wall of the auditory vesicle initially consists of monostatal cubical epithelium. In human embryos about 5 mm long, however, the medial wall of the vesicle is already thicker in the region adjacent to the auditory ganglion. It is at this site that the peripheral processes of the ganglionic cells come into close contact with the epithelium of the wall of the auditory vesicle. This epithelial intumescence represents the first appearance of all the future terminal organs of the labyrinth, the so-called macula communis. It grows together with the vesicle, and when the latter divides, the macula communis also divides into two sections (superior and inferior) which remain connected for some time by an epithelial "bridge" of characteristic structure. Following replacement of this bridge by typical indifferent epithelium from the wall, two neuroepithelial maculae are formed, one located in the pars superior and the other in the pars inferior (Alexander, 1900). The second of these later moves to the medial and anterior area of the auditory vesicle, while the other (located in the pars superior) is shifted by the growth of the latter to the lower and external walls. The rudiment of the terminal organs, located in the pars superior, gives rise to the utricular auditory macula (macula utriculi) and the ampullar crista (crista ampullaris) of the anterior vertical and lateral (horizontal) semicircular canals. The rudiment of the terminal organs in the pars inferior gives rise (inside and posteriorly in the ampulla) to the posterior vertical canal, forming its auditory crista (crista ampullaris posterior). The remaining portion of this rudiment grows lengthwise and divides into two rudiments, a small one above and a large one below. The former becomes the saccular auditory (macula sacculi), while the latter continues to develop and forms the rudiment of Corti's organ (papilla basilaris cochlea). In lower vertebrates, the auditory macula of the lagena (macula lagena) also develops at the outer end of this epithelial rudiment (cf. Alexander, 1920; Fischer, 1922). /71

However, the ontogenesis of the individual auditory maculae and cristae has not been conclusively explained at the present time. Thus, the genesis of the ampulla of the posterior vertical semicircular canal appears to be extremely complex, since the canal itself comes from the pars posterior while its auditory crista develops from the neuroepithelium of the pars inferior. The development of all receptor structures of the labyrinth from one rudiment (the macula communis) with subsequent division into the corresponding parts explains the fact that in many adult animals the individual receptor structures of the labyrinth are connected by strands of epithelium with particular structures. Occasionally, the topography of the distribution of these receptor structures within the labyrinth is disturbed and the individual sensory macula can move to unusual lo-

cations; this is especially characteristic for the macula neglecta, which normally is formed from the connection between two different receptor maculae (Krause, 1906; Streeter, 1907; de Burlet, 1934).

The development of the labyrinth is subject to certain variations in representatives of the different classes of vertebrates. Thus, for example, according to the data of Noorden (1883) and Wenigs (1911), in teleostean fishes the anterior vertical canal develops first, followed by the posterior vertical and then the horizontal canal, which Wenigs relates to the later appearance of the horizontal canal in phylogenesis. In a number of tailless amphibians (for example, *Rana*, *Bombinator*, etc.) the horizontal canal appears first, followed by the anterior and finally the posterior vertical canal. However, in another member of this order (*Bufo*) the anterior vertical canal develops before the horizontal one. In birds, the auditory vesicle is completely separate from the ectoderm, while the recessus labyrinthi and ductus endolymphaticus are formed by fusion of the cranial walls of the vesicle (Fuchs, 1923).

Differentiation of the epithelium of the auditory maculae into receptor and supporting cells is observed in human embryos 20-30 mm long and is complete in 70 mm embryos (Kogan, 1952). The otolith membrane is formed by the secretory activity of cells. The first otoliths appear in embryos 55 mm long. The dimensions of the otoliths increase visibly in subsequent stages of development (Kikuchi, 1934).

During the growth of the labyrinth, the sensory epithelium of the maculae and cristae retains its thickness completely, while the surrounding walls become much thinner. In this multiple epithelial layer, one can distinguish peripherally located large cells with round nuclei (future receptors) and narrow supporting cells located further down. On the surface of this epithelium, covering plates are visible, between which the heads of the receptor and supporting cells are located. According to Held (1902), each of these cuticular plates contains a diplosome, from which the inner and outer flagella develop in each cell. The sensory hairs (or rather, groups of fine hairs) develop shortly before the diplosome; a gelatinous substrate then develops beneath the sensory epithelium of the macula. Kolmer (1927) suggested that this gelatinous substrate may arise as the result of a secretion similar to the chitin of arthropods. Differentiation of receptory epithelium, according to Kolmer, proceeds from the center toward the periphery; on the periphery of the macula and crista it is often possible to see mitoses in the cells even at the latter stages of development, when the mitoses are completely absent from the centers of these structures. The increase in the dimensions of the sensory structures of the vestibular apparatus first takes place by cell division, and later exclusively by an increase in volume of the individual cells. Kolmer mentions that the phylogenetic recapitulation of form, which we encounter in the ontogenesis of higher vertebrates, does not show up as markedly in any other organ as in the growth of the labyrinth. /72

In 1912 Studnicka described the process of formation of the membranes covering the inner ear. According to his description, a great many fine lateral growths form on the hairs or elements of the hair cells; these growths are connected to form a gelatin-like exoplasm. Studnicka observed this process during the development of the labyrinth of the lamprey and took it to be the prototype for the development of the covering membrane in all vertebrates. Later, Wittmaack (1926) assumed a similar process for mammals. However, several later observations have shown that he did not have sufficient grounds for doing this.

The first result of the formation of the covering membranes of the macula and crista takes the form of a very thin gelatin-like layer which lies above the surface of these structures. In man, this occurs at the beginning of differentiation of the macula communis. At this stage, the epithelium of the terminal organs is very compressed and the end plates are very narrow elements, 1-2 μ wide, linked by locking plates. In each such polygonal section, one can distinguish a diplosome and sometimes the flagellum as well. At the start, the gelatinous mass covers all the cells of the rudimentary structure uniformly, but by the beginning of the third lunar month in man it is possible to see fine gelatin-like threads running from this layer to the heads of the supporting cells. As the development of the cupola or otolith membrane proceeds, the free spaces above the heads of the supporting cells become more and more evident; it is in these spaces that the short sensory hairs will first be located. At subsequent stages of development, the gelatinous substrate develops further and cylindrical spaces are formed around the sensory hairs in it. In the macular membrane their length is almost equal to the length of the cell, but in the cupola they exceed it several times.

Formation of otoliths in all vertebrates is observed when there are only slight traces of the gelatinous substrate and when no traces of secretory activity are observed in the sensory epithelium (Herzog, 1925; Kolmer, 1927). According to data from the latter authors, increased concentration of calcium salts is observed in the region of the macula communis; these salts precipitate out of solution on the albumin grains found in the gelatinous substrate, which serve as centers of crystallization, here forming compounds of albumin with calcium carbonate. Initially, all of the otoliths are the same size; it is only at later stages of development that there is a difference in their sizes, when the utricle contains large, small and medium-sized otoliths (de Burlet, 1934; Lorente de No, 1931).

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In the Cyclostomata, the otoconia are initially separate and later combine into one "stone" (in Myxine, they remain individual); in other fishes there are a very great many centers of crystallization, resulting in the formation of fine otoconia.

In teleostean fishes, there are three centers of formation of

coarse otoliths. The latter are composed of concentric layers and increase in size as the fish grows during its entire lifetime; the number of layers corresponds to the age of the fish (Immermann, 1907). Dark and light layers can be distinguished in these otoliths: the dark layers are richer in organic substances and are deposited in the winter months, while the light layers are rich in aragonite and are deposited mainly in summer.

In reptiles, the developing otoliths have the appearance of crystals or crystal-like bodies with rounded corners. They are precipitated from a concentrated solution and form a viscous mass bound by a gelatinous substrate. This is also characteristic of the calcareous mass in amphibians, which not only is formed above the macula, but moves out of the sacculus into the ductus and sacculus endolymphaticus (and in Anura, into a branch of the ductus), reaching the spinal cord and forming (at the level of each spinal ganglion and adjacent to the latter) growths which produce a system of calcareous vesicles. The function of the latter remains unknown at the present time (Herter, 1922).

In birds, the otoconia initially develop in the form of numerous crystals of aragonite, arranged in several layers. The stratification of their structure is especially marked in the otoconia of the macula lagena.

In mammals, the development of the otoliths begins relatively early (when the human embryo is about 48 mm long, for example); they are seen as separate long structures on the surface of the gelatinous covering of the macula. The otoliths are shaped like prisms with built-up pyramids. They grow very slowly and do not exceed 5 μ .

In 1960 Belanger studied the postnatal development of the otolith membrane in the rat (3 to 9 days old). Histochemical, autoradiographic, and X-ray tests were conducted. The data indicate that the otoconia in the rat are heterogeneous crystalline bodies. They grow by the accretion and combination of fine particles and subsequent lamellar arrangement. They contain calcium, perhaps phosphorus, and neutral and acid sulfates of mucopolysaccharides. All of these substances are concentrated at the periphery. According to this author's data, the otolith membrane consists of neutral and acid sulfates of mucopolysaccharides, but in different proportions. There are no mineral layers in its composition.

Sudden and relatively massive growth of crystals is characteristic of the growth process of the otolith apparatus in the chick. No traces of them are visible on the 6th day of incubation, even in studies conducted with double refraction; in several hours, however, a large amount of lumpy precipitate is visible. According to Herzog (1925), it is entirely probable that this is a process of crystallization which occurs at the moment the required concentration of salts in the fluid is reached. With the aid of plasmatic substance,

regardless of whether it is formed by secretion or by decomposition /74
of the cells themselves; the content of calcium salts steadily rises in the fluid; at the same time, the viscosity increases, i.e., a process of gelatinization takes place. As a result of these two processes, crystallization spreads over the entire gelatinized area. When the calcium is removed with acid, a fibrillar reticulated structure perforated with holes remains; these holes retain the shape of regular crystals (Herzog, 1925). The latter stages do not show any further changes. The layer of crystals grows as the individual crystals increase in size.

The appearance of otoliths at different stages of embryogenesis evidently may have some connection with the earlier development of the otolith apparatus relative to other receptor formations of the inner ear and its earlier inception of activity (Obraztsova, 1961).

Approximately in the middle of the 19th century, Lang (1863) described certain cells, equipped with cilia, in the auditory vesicles of Cypriniformes. These cilia were later studied by Hasse (1869), working with live fish, reptiles, and domestic fowl. According to both authors, these cilia terminate in fine canals which traverse the gelatinous substance of the cupola. Kolmer (1927) later confirmed the findings of Lang and Hasse and showed that similar relationships exist in the ear of man and other mammals. Bowen (1932) viewed the cupola as an artifactitious structure, the result of the action of fixators producing condensation of endolymph around the crista. However, he described motile cilia, and his observation of their motion led him to conclude that there cannot be any substance between the cilia which is capable of limiting their motion. Witmaack (1926) thought that the cupola was formed by the secretion of ectoplasm from the cilia, while Shambaugh (cited in Werner, 1940) studied newborn primates and observed that the cilia lie in canals which run perpendicular to the surface of the epithelium. Van der Stricht (1921) held that the cupola is a cuticular formation that develops from subjacent cells arranged in parallel prisms joined by a cement, among which the sensitive hairs arise. Dohlman (1960) studied the operating mechanism of the cupola and observed that the gelatinous substance reaches the opposite walls of the ampulla.

Vilstrup (1950) showed that the ampullar cupola is formed from the sensory epithelium of the crista in the following way. Long protoplasmic growths extend from both rows of cells in the sensory epithelium, protruding far out into the ampullar space. Here they fuse to form a reticular syncytium. The individual cell nuclei assume an irregular elongated shape; the nuclear chromatin blends with the general mass, losing its structure, and migrates into this network. Beyond the limits of the epithelium, these nuclei assume a circular or oval shape. In this manner, a subcuticular zone is formed. The fibrillary zone, in Vilstrup's opinion, is formed from the network of the syncytium. Vilstrup thinks it possible that the protoplasmic strips and nuclei, forming the cupola and having the appearance of a syncytium, result from previous mitotic division of

the nucleus which occurred in the epithelial cells, and therefore the material encapsulated in the endolymphatic space also contains daughter cells.

On the other hand, Savin (1960), unlike Vilstrup, believes that the formation of the cupola in the chick embryo occurs without cellular migration in the direction of the endolymph and that the cupola appears above the crista without the transfer of any kind of material whatsoever from the epithelium of the crista to the cupola. He considers the above-mentioned cilia to be protoplasmic growths which do not contain nuclei. The auditory vesicle appears on the 6th or 7th day of incubation, and the cupola begins to form over it; this takes place at a time when there has still not been any division into receptor and supporting cells in the epithelium of the cupola. The histogenetic process in the cupola takes place on the 7th through 14th days of incubation; during this period, the author failed to observe any of the processes described by Vilstrup.

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After studying the development of the auditory vesicle Böttcher (1869), Alexander (1900), Krause (1906), Streeter (1918) and Kolmer (1927) all stated that the common origin of all receptory formations in the inner ear is the macula communis (macula acustica). However, Lorente de No (1931), using special neurohistolytic methods to study the development of the innervation of these receptor structures, came to the conclusion that only three cristae and a portion of the utricular macula develop from the macula communis. The remaining portion of the utricular macula, saccular macula, and cochlea develop from a rudiment that appears later. The basis for this conclusion, according to their data, is that the fiber of the 8th nerve is still outside the epithelium when the macula communis appears, as established in their day by Retzius (1892), Held (1926), Cameron and Millingan (1910), and Tello (1931). In the opinion of Lorente de No, the termination of the fibers in the epithelium is secondary, and this process, according to the data of a number of authors (Lorente de No, 1931; Krause, 1906; Ramon and Cajal, 1909; Bielschowsky and Brühl, 1908), takes place very late. These fibers undergo considerable ramification beneath the epithelium and form a mesh which Ramon and Cajal (1909) called the plexus externus and from which numerous fibers then extend into the epithelium, which is still in an undifferentiated state. According to Bielschowsky and Brühl (1908), the epithelial cells must reach a certain stage of development in order for the growing nerve fibers to come into contact with them. After such contact is established between the nerve fibers and the receptor cells, many of the nerve fibers which penetrated the epithelium earlier atrophy. This occurs at a very advanced stage, and traces of resorption of nerve fibers are still evident in newborn animals. On the basis of the data presented by Lorente de No regarding the development of the innervation of the inner ear in mouse embryos, we can conclude that this process does not take place simultaneously in all receptor formations of the inner ear. He shows that at the time that the nerve fibers have already penetrated the receptor epithelium of the utricular macula,

they have still not penetrated the receptor epithelium of the sac-
cule, although they have formed the plexus externus beneath the sac-
cular macula. The receptor epithelium of the cochlear canal is
still not innervated at this time.

Differentiation of the receptor epithelium of the utricular
macula does not occur simultaneously in all parts of it. The area
 $a \pm b$ (in Lorente de No's notation) is the first to be differenti-
ated, while area c is differentiated at later stages. The saccu-
lar macula is still not developed at this time, and its differenti-
ation takes place later on. Lorente de No (1931) observed that the
nerve fibers in still undifferentiated mesodermal tissue lasted un-
til the protrusion of the auditory vesicle and only then was the
sacculus formed. Then the plexus developed beneath its wall; only
after this was any growth and differentiation observed in the recep-
tor epithelium of the saccular macula. This author feels that the
dual innervation of the sacculus is achieved mechanically by the
special method of its development, since the nerve fibers, as they
grow forward, pass through it partly upward and partly down. The
plexus which is then being formed lies immediately next to the ves-
tibular ganglion (Scarpa's ganglion), and when this growth forces
the ganglion backward, the fibers of the saccular nerve (nervus
saccularis) divide into two bundles. The crista (according to the
data of the same author) is supplied by only a single nerve, since
the crista itself at this time consists only of thickened epitheli-
um. In mouse embryos 11 mm long, intense proliferation of undiffer-
entiated cells occurs at the center of the crista and the nerve fi-
bers are also divided into two bundles. At the same time that dif-
ferentiation of the sensory epithelium is taking place, the nerve
fibers reach the hair cells. At this time, a protrusion appears in
the center of the crista, dividing it into two isolated halves, each
of which has its own nerve. One of these halves corresponds to the
canal side, the other to the utricular side. In mice, this protrusion
atrophies later on and the crista again becomes essentially a
single structure. However, the independent innervation of the two
halves of the crista is retained. Lorente de No draws the follow-
ing conclusions from these personal observations: /76

All receptor organs (terminal organs) of the inner ear are com-
plex structures. Each part of the receptor organ, distinguished by
a particular structure and innervation, has its own separate repre-
sentation at the center with a definite point of location. Proceed-
ing from the complex structure of the utricular macula and the re-
gional differences in the otolith membrane, Lorente de No proposes
that both maculae function differently. Thus, the region of the
utricular macula bears a great resemblance to the crista, all the
more so because the otoliths lie on it, while area c shows a simi-
larity to the saccular macula. In another of his studies, Lorente
de No (1937) showed that the fibers connected with the latter two
structures show parallel subdivisions in the nerve centers.

Some papers indicate that a few outgrowths of the ganglionic

cells near the ganglion penetrate into the utricular space and are detected there as "suspended" free nerve endings (Chernyakhovskiy, 1928; Lorente de No, 1926; Kogan, 1952). These aberrant or provisory fibers disappear during further development.

In 1958, Batten studied the development of the acoustic ganglion in sheep. According to his data, the auditory ganglion arises from placodal cells that separate from the auditory vesicle (in sheep fetuses 6 to 14 mm long). In this regard, Batten takes the generally accepted view that they develop from a common acoustico-facial neural crista. After the 8 mm stage, the separation and migration of the cells of the placode become more energetic. The auditory ganglion is closed after the ganglia geniculata. However, these ganglia retain their differences in cytological differentiation and he stresses the fact that the g. geniculata contain young neuroblasts until such time as the rudiment of the auditory ganglion becomes noticeable. Contact between the rudiments of the two ganglia gradually decreases after the 11 mm stage, i.e., when the cells of the placode begin to differentiate into acoustic neuroblasts. As a result of his investigations, Batten comes to the conclusion that the auditory ganglion arises exclusively from placodal cells. The definitive vestibular ganglion arises from the pars superior along with the lateral zone of the pars inferior, in which early neuroblasts appear. The definitive cochlear ganglion develops somewhat more laterally, formed from the remaining medial portion of the pars inferior with the addition of the future diffuse placodal portion of the basal vesicle.

Morphological Studies of the Development of Corti's Organ

The processes of differentiation of the elements of the cochlear canal begin in the basal portion and gradually progress upward (Böttcher, 1869; van der Stricht, 1918-1922; Alexander, 1926; Held, 1926; Kolmer, 1927; Weibel, 1957). The differentiation processes in the cochlea are complete 10 to 14 days after an animal is born, but the overall growth of the organ continues (Kolmer, 1927). The differentiation of Corti's organ in the basal portions of the cochlea coincides with the beginning of its functioning. In a series of interesting morphophysiological papers on the fetuses of a species of opossum (*Didelphis virginiana*), McCrady and his coworkers (McCrady, 1933; Larsell et al., 1935; McCrady et al., 1937; Larsell et al., 1944) were able to demonstrate a complete correspondence between the process of histological differentiation of the cochlea and the ability of the fetus to detect various auditory frequencies. The latter was determined with the aid of biocurrents detected at the round window. Thus, at the time when Corti's organ is developing in the lower helix, i.e., from the 2nd to 59th day of the fetus' life in the womb, the fetus can detect audio frequencies from 500 to 6000 Hz. Later, and only after development of the middle and upper helices (beginning on the 59th day), the fetus can detect audio frequencies from 300 to 10,000 Hz; on the 68th day, it can detect 200

to 2000 Hz. According to data presented by Mazo (1958), the establishment of the function of the auditory apparatus in rabbits is accompanied by the formation of "islands of hearing" at individual frequencies, which subsequently blend together.

At the beginning, the cochlear canal grows in the shape of a narrow tube with an oval cross section, whose epithelium very soon differentiates into two distinct morphological forms: high cylindrical epithelium at the base and low cubical epithelium at the apex of the cochlear canal. The high epithelium at the base later becomes Corti's organ. The low epithelium at the apex becomes Reissner's membrane and the covering of the lateral internal wall. Two auxiliary thickenings or toruses then develop on the high epithelium at the base: the larger is the axial torus and the smaller is the lateral torus. The nuclei in the cells of these toruses move closer to their bases. The cells located between these toruses are markedly vacuolized. The subjacent connective tissue thickens to mark the beginning of formation of the basilar membrane.

The cytoplasm of the flattened epithelium in the future Reissner's membrane also acquires numerous vacuoles. The connective tissue beneath the epithelium, which forms the scala vestibuli later on, becomes separated.

Soon the lumen of the cochlear canal acquires a triangular shape as a result of the development of the scala vestibuli and the scala tympani, which are filled with perilymph. It is at this time that the most intensive differentiation processes occur in the region of the future Corti's organ. Against the background of lightly stained vacuolized cells in the basal cochlea, located between the two toruses mentioned above, transverse sections reveal two cells with intensely stained cytoplasm (Kolmer, 1927; Weibel, 1957) and highly organized nuclei. Their long axes are arranged at angles to one another. The bases of the cells do not reach the basilar membrane. This is the beginning of the differentiation of the external and internal hair cells (Weibel, 1957). Later on, it is possible to distinguish among them the beginnings of the future inner rods, which appear as bright vacuolized elements with a broad base, located on the basilar membrane. At this stage, it is possible to distinguish 6 rows of supporting cells whose nuclei are located close to the base and whose cytoplasm is vacuolized. The first row becomes the supporting elements for the internal hair cells; the second row becomes the above-mentioned inner rods; the third row becomes the outer rods, and the rest become Deiter's cells. /78

On the apical surfaces of the hair cells, one can see intensely stained disks, which protrude above the level of the other cells. This type of convex disk is also called a hair disk (Held, 1926), because a tuft of short hairs connected to diplosomes (van der Stricht, 1918) soon develops on its surface. The bases of the cells are distended somewhat. Between them and the subjacent Deiter's cells is a rimose space filled with fluid.

Soon both types of rod cells begin to divide rapidly against the general background of the other supporting cells. The heads of the outer rod cells are very closely linked to the tops of the inner rod cells. Within the inner rod cells one can distinguish a fibrillar bundle drawn from the nucleus (located at the base of the cell) toward the top, where they form the fibrillar system of the outer rod cells in the head region. The rod cells are oriented at a characteristic angle of 45° to one another.

The phalanxes of Deiter's cells are arranged in three parallel spiral rows. Their apical ends pass between the hair cells, reaching the tops of the latter. Here they are connected with the fibers of the rod cells and the adjacent cell phalanxes. In this manner, the reticulate membrane is formed, which contains the tops of all the hair cells. The canal of the antrum of Newell is formed by the shifting of the rimose spaces between Deiter's cells and the outer hair cells. This occurrence is apparently not connected with the cytoplasm of the cells, as van der Stricht thought, but with their movement. As the overall growth of the organ proceeds and the basilar membrane thickens, the space between the rod cells expands, resulting in the formation of a tunnel (Weibel, 1957). An analogous tunnel is formed between Deiter's cells and Hensen's cells. The filling of the tunnel with fluid may possibly be the result of secretory activity of embryonal secretory elements including the inner rod cells (van der Stricht, 1919). Beyond these are the low cells of Claudius, which develop from the most lateral part of the basilar papilla (van der Stricht, 1919; Weibel, 1957).

The growth of the spiral limb is connected with the thickening of the connective tissue fold which lies on the lamina spiralis ossa. As we know, the tectorial membrane is connected to the epithelium which covers the spiral limb. This epithelium grows from the inner part of the above-mentioned large torus, the basilar papilla. The epithelium forms unusual radial disks with a transverse arrangement of elements in 18-16 rows. The bases of the epithelial cells, together with their nuclei, are embedded deep in the subjacent connective tissue, where they combine to form a palisade.

Between the spiral limb and the other elements of the basilar torus there develops a depression, the inner spiral recess (Sulcus spiralis internus), which is initially covered by high epithelium and later by flattened epithelium. Above it is the tectorial membrane, which in the opinion of Weibel (1957) is formed essentially from fibrils which develop on the surface of the cells in the epithelium of the spiral recess. Held (1926) suggests that the tectorial membrane is formed by the fibrillar and not the cuticular transformation of the apical surface of all the supporting elements, with the exclusion of the auditory cells. He noted how the membrane separates from the subjacent epithelium in the region of the spiral recess at later stages of development. This separation then spreads to the area of the inner and outer hair cells, so that the tectorial membrane covers their surface; this is of great significance for the

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future functioning of the organ, since the hairs appear at the apical surface of the hair cells only after the formation of the tectorial membrane.

Kölliker (1855, 1861) viewed the tectorial membrane as a cuticle. Böttcher (1869), on the other hand, was of the opinion that it developed from the apical cell growths of both thickenings or toruses of the basilar papilla. He anticipated the studies of Held and Weibel in this regard. However, Böttcher also felt that the formation of the tectorial membrane involves the participation of the hair cells, whose hairs penetrate directly into its fibrillar core and are broken off during fixation and decalcification of the organ. These incorrect ideas are held even today. For example, Wittmaack (1918, 1924), Plotz and Perlmann (1955) agree with Böttcher on this point, claiming that the hairs of the receptor cells and the fibrils of the tectorial membrane constitute a whole, so that the membrane is attached to their apical surface as well as to Hensen's cells.

The vascular stria develops from the elements of the roof of the cochlear canal and occupies the lateral portion of the wall. It arises from a unique combination of the vascular epithelium and connective tissue. In the opinion of Katz (1890), Hann (1906), Kolmer (1921, 1927), Fieandt and Saxen (1936-1937), the elements of the vascular stria have an exclusively epithelial origin; capillaries from the spiral ligament actively grow into its multilayered epithelium. On the other hand, Shambaugh (1907) and Weibel (1957) hold that the development of the vascular stria, besides involving the capillaries, also involves the participation of mesenchymal elements which grow into the epithelium like the capillaries; however, the epithelial cells, after passing through the basilar membrane, actively send their processes into the deep-lying connective tissue and bind the vessels together with a layer of mesenchyma. Individual epithelial cells may separate from the epithelial layer and become embedded in the connective tissue. Hence, there is always a layer of mesenchymal elements between the epithelium and the wall of the vessel.

In human embryos in their 5th month of intrauterine life, the vascular stria is completely developed and contains a large amount of glycogen, as Kolmer (1927) was the first to point out. In the latter's opinion, the glycogen gradually migrates into the endolymph and disappears with the advancing differentiation of Corti's organ. However, later histochemical studies (Vinnikov and Titova, 1961; Falbe-Hansen and Thomsen, 1963) have shown that it is actually retained in elements of the definite organ, including the vascular stria. Its absence at later stages is evidently related to the decalcification of the cochlea at later embryonal ages as well as in adult animals.

Immediately adjacent to the vascular stria is a narrow strip, the prominentia spiralis. Its development is very similar to the

development of the vascular stria. As the growth of the organ proceeds, the growths from the cells in the outer spiral recess penetrate ever more deeply into the subjacent connective tissue. When they separate, individual epithelial cells may enter the subjacent connective tissue, forming distinct "protoplasmic spheres" there. The "spheres" may be located in the protoplasm of cells, or they may be isolated (Weibel, 1957). Fieandt and Saxen (1937) report that even at these stages, in these areas of the membranous cochlea, processes of resorption of tissue detritus are in progress; this substance gradually comes to form part of the endolymph. /80

Nerve elements of the labyrinth are observed in the human embryo as early as the 30-somite stage, corresponding to a length of 4-5 mm. At the anterior medial wall of the auditory vesicle, there is an accumulation of ganglionic cells, together forming the rudiments of the auditory and facial nerve ganglia. The origins of the development of these ganglia are not completely understood even today. Some investigators believe that they are basically developments from the ganglionic disk, and that (in particular) the embryonal cells of the auditory ganglion are formed simultaneously from the wall of the embryonal brain, from the epithelium of the auditory vesicle itself, or even from the ectoderm immediately adjacent to the auditory fossa (Streeter, 1918). In recent years, however, more and more researchers have come to accept the view, based on xenoplastic transplants, that the cells of the auditory ganglion originate (either to a significant extent or completely) in the auditory vesicle (Niessing, 1932; van Campenhout, 1935; Groth, 1939; Theiler, 1949).

Formation of the ganglion is not the same in all classes of vertebrates. This has produced considerable confusion and vagueness regarding the origin of the ganglionic cells. De Burlet (1934) feels that the material for formation of the ganglion may be provided by the nerve tube, the ganglionic disk, or the placode from which the auditory vesicle is formed. These components participate in the formation of the ganglion to varying degrees in different classes of vertebrates.

The facial nerve ganglion separates from the auditory ganglion very early. The latter retains a close interrelationship with the auditory vesicle. After separation of the latter into two parts, the rudiments of the ganglion undergo the same division, and its lower (or cochlear) portion forms the ganglion for the macula sacularis and the crista ampularis of the superior vertical canal. In addition, the lower portion contributes to the formation of the cellular mass of neuroblasts from which the spiral ganglion is differentiated; the latter is first found in human embryos at the 9 mm stage.

When the cochlear canal undergoes helical torsion, the mass of neurons located on the axis of this spiral likewise undergoes helical torsion. Their peripheral endings form Corti's organ, while

the central endings form the cochlear nerve.

Hence, the histogenesis of the membranous labyrinth may be considered to have been well covered by modern histological methods, although of course the histogenesis of the auditory portion of the labyrinth has been investigated in greater detail than the histogenesis of its vestibular portion. However, the available data (with the exception of the research performed by McCrady et al.) provides no basis for establishing the cytochemical and histophysiological processes which are related (on the one hand) to the plastic processes of differentiation and (on the other hand) to the beginning of functioning of Corti's organ.

Cytochemical and Electron Microscope Studies of the Development of the Inner Ear

We undertook an investigation of the embryo- and histogenesis of the inner ear in representatives of various classes of vertebrates for the purpose of studying not only the morphological, but primarily the cytochemical changes which occur during the differentiation of the receptor structures of the inner ear. It was not possible for us to investigate this entire complex of biologically active substances, which we have the ability to detect by histochemical means, in all representatives of the different classes of vertebrates. In a number of cases the obstacle was the impossibility of having directly, in the laboratory, living young in successive stages of development; on other occasions, the problem lay in the purely technical difficulties involved in intravital isolation of the inner ear owing to the small size of the embryos and larvae (in the loach, for example). On still other occasions the problem lay in the poor and highly nonuniform permeability of the tissues with respect to fixing agents or histochemical reagents at various stages of embryonal development.

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The most suitable (and hence the most thoroughly studied) material proved to be the internal ear of the chick, in view of the ease of obtaining the material in the laboratory at all stages of embryonal development.

However, from our viewpoint, this circumstance does not exclude the possibility of obtaining an answer to the questions we have set ourselves, because the process of morphological and chemical differentiation of the receptor structures of the inner ear takes place in generally the same manner in all of the vertebrates we have studied, showing (as we shall see below) the very same regularities in the changes of the distribution and degree of concentration of the biologically active substances which we studied.

DEVELOPMENT OF THE INNER EAR IN GANOID FISHES

As a glance at the references will show, data on the embryogenesis of the inner ear of ganoid fishes are lacking in the available

literature. Neither did we encounter any data on the histochemical investigation of their histogenesis.

Three days before they are due to hatch from the egg, sturgeon embryos still possess a non-differentiated auditory vesicle, in which one can detect the formation of the common source of all future receptor structures of the inner ear--the Macula communis. Between the auditory vesicle and the brain, adjacent to the wall of the former, is an accumulation of minute cells, forming the rudiment of a ganglion (Fig. 1). In preparations stained with silver, it is apparent that the cells located on the periphery of the ganglion already have processes that stain with silver, extending to the wall of the auditory vesicle.

The auditory vesicle at this stage consists of high epithelium 2-3 rows thick. All of the cells in this epithelium are more or less the same. They contain some kind of large argentophyllic granules. In preparations stained with silver, however, it is possible to see cells with dark and light nuclei. In analyzing the auditory vesicle for nucleic acid, individual round, larger and brighter nuclei are seen. The DNA concentration in these nuclei is average. The nuclear membrane of the majority of cells shows a positive reaction to DNA in all methods of preparation. We may assume that there is an adherence of fine bits of chromatin to the nuclear membrane; this is a purely theoretical assumption, however, and the immersion objectives of optical microscopes fail to reveal any such bits. The maximum reaction to DNA is shown by the chromatin of dividing cells, which are found in rather large numbers at this stage. Also rich in DNA are the nuclei of the mesenchymal cells surrounding the auditory vesicle. It is interesting that the nucleoli in the nuclei of the cells in the wall of the auditory vesicle have a lower concentration of nucleic acid than the chromatin of the nucleus. The same applies to both the DNA and RNA of the nucleoli. The RNA concentration in the cytoplasm of these cells is average and is uniformly distributed over the entire thickness of the epithelial layer. /82

In the adjacent rudiment of the ganglion, the nuclear chromatin gives a very mildly positive reaction to DNA. The nucleolus stands out sharply against this background; the maximum concentration of DNA is on the periphery of the nucleolus and a very large concentration of RNA is at its center. In Fehling's reaction, however, the perinucleolar region of the nucleolus stands out much more brightly; from this we may conclude that the maximum coloration of this portion of the nucleolus in a reaction with galloxyanin depends on the presence here not only of DNA but of RNA as well. The reaction has a uniform intensity in the chromatin grains in the Fehling reaction and in the galloxyanin reaction. The concentration of RNA in the cytoplasm of these cells is the same as in the cells of the wall of the auditory vesicle. Among these fine cells of the rudimentary ganglion, we find individual, larger cells having a large circular nucleus with completely clear karyoplasm and very clear (i.e., poor in DNA), fine-grained chromatin. The outlines of these nuclei are

fine and bright. Each of these nuclei contains 2 nucleoli, with very dark edges and a brighter center (when prepared with gallocyenin); this agrees with the description of the nucleoli of smaller cells.

The reaction for total (or general) protein in the wall of the auditory vesicle of the sturgeon, 3 days prior to hatching, revealed the existence of protein-rich lumps of yolk and smaller granules of some pigment. The cytoplasm and chromatin of these cells are marked by a moderate concentration of protein and the carboxyl groups of protein molecules. Their distribution is remarkable for its high uniformity over the entire thickness of the epithelial layer, as well as the distribution of the thiol groups, which have a weak concentration (Fig. 2).

In the cells of the rudimentary ganglion, the highest concentration of protein and carboxyl groups of protein molecules is found in the nucleus and nucleolus of the larger ganglionic cells. In the cytoplasm, the concentration of total protein and carboxyl groups is average. The concentration of thiol groups in all of these cells is very low.

When the fry hatch, the auditory vesicle is divided into the Pars superior and the Pars inferior. Division of the Macula communis takes place at the same time. However, the rudiment of the receptor epithelium separates from the epithelium of the wall of the auditory vesicle only by the multiplication of its component elements and not by their formation. A significant number of lumps of yolk and pigment grains are found in the wall of the auditory vesicle. In preparations treated with osmium, the cells of the rudiment of the sensitive epithelium appear to be highly vacuolized, especially in the apical areas. In individual cells, the cytoplasm appears darker and more homogeneous.

However, during the first days after hatching, significant changes occur in the structure of the auditory vesicle: its configuration becomes more complex; the rudiment of the endolymphatic canal is formed; development of the semicircular canals and the rudiments of the maculae and cristae takes place. Around the developing inner ear, the cartilaginous capsule begins to develop. In the rudiments of the receptor epithelium, especially the rudiments of the maculae, there is a division of the cellular elements into receptor and supporting structures: the former are located in the upper third of the epithelial layer and are distinguished by their larger, more circular nuclei, poor in chromatin; the latter (supporting cells) are located mainly in the basal portions of the epithelial layer and have smaller, oval nuclei rich in chromatin.

The ganglion adjacent to the auditory vesicle consists primarily of small, still incompletely differentiated cells. However, they show numerous nerve fibers (well impregnated with silver) which grow into the rudiment of the sensory epithelium; however, it is still impossible to detect the formation of synapses in the

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sensory cells.

The highest concentration of nucleic acid is found in the apical cytoplasmic portions of the epithelial layer. The DNA concentration in the nuclei of the receptor cells becomes much weaker than initially. The former concentration is retained only in the nucleolus. The nuclei of the sensory cells have a quite sharply defined membrane, in which a light green and pink coloration can be seen when they are treated with methyl green-pyronine; this coloration may have something to do with the close proximity of both DNA and RNA particles. It is quite possible, however, that this impression is the result of superposition. A somewhat higher concentration of DNA is found in the nuclei of the supporting cells. All that has been stated above applies to the rudiment of the sensory epithelium of the macula. The rudiment of the sensory epithelium in the cristae of the vertical canals differs somewhat in its development from the macular tissue, and here there is only a morphological separation of the sensory cells; as far as their cytochemical characteristics are concerned, however, this is less marked and all the cells of the rudiment of the crista appear identical as far as the distribution of the nucleic acid is concerned.

In the subjacent ganglion, the concentration of nucleic acids in the nucleolus is reduced somewhat. Their distribution in the nuclear chromatin and cytoplasm remains constant, however.

At the moment of hatching, the cells of the rudimentary sensory epithelium are remarkable for their very high concentration of total, or general, protein. The outlines of the nucleus and cells can be distinguished with difficulty against the common dark background. Visible on the surface of the rudimentary sensory epithelium is a flat disk with the maximum concentration of protein, separated from the apical surface of the cells by a brighter space. This is evidently the site of future gelatinous structures. In the cavity of the auditory vesicle, on the surface of the sensory epithelium, there are some spherical accumulations whose configuration is similar to that of a mulberry; they contain an average concentration of total protein, and may possibly be granules of some secretion.

At the end of the first day of growth, a decrease in protein concentration is observed in the formative maculae, both in the nuclei and in the cytoplasm of the cells in the receptor epithelium. The original concentration of protein is retained only in the nucleoli, which stand out sharply against a generally brighter background.

The concentration of total protein in the cells of the rudimentary ganglion increases significantly toward the end of the first 24 hours, but its distribution remains uniform. Its concentration is the same in the chromatin, nucleolus, cytoplasm of the bodies of the cells, and in the nerve fibers proceeding from the latter. It is interesting that a high concentration of carboxyl groups is retained in both the formative rudimentary maculae and in the cristae.

One fact should be mentioned which has come up repeatedly in connection with the embryos of still other vertebrates: the differentiation of cellular elements can be seen first of all in the reaction of protein molecules to carboxyl groups. Differences between individual cells and tissues also show up most sharply in this reaction. In the reaction to carboxyl groups, the first thing to be noticed is the difference between the cells in the central portion of the macula, which are beginning to differentiate, and the cells in its peripheral areas, which are still undifferentiated, in the rudimentary macula. The cells in the central portion appear brighter, since the concentration of carboxyl groups in them is lower. A difference can be seen in the concentration of carboxyl groups between individual cells, arranged in rows, and even within a single cell. The largest concentrations are found in the apical and basal portions. The highest and most uniform concentration occurs in the supporting cells and in the undifferentiated cells of the peripheral portions of the macula. The maximum concentration of carboxyl groups is seen in the region of the basilar membrane. /84

When subjected to the reaction for carboxyl groups, the ganglion of the eighth cervical nerve reveals an unusually clear structure and a high content of carboxyl groups. There are, however, differences in concentration of carboxyl groups between the individual ganglionic cells. The branches extending from the latter, their pathways to the brain and into it, where separation of the neurons is seen, are all clearly visible. It is very difficult to say anything definite with respect to the thiol groups, since their concentration remains very low at this stage.

Differentiation of the sensory epithelium continues into the second day after hatching, as it does in the rudimentary ganglion. In the latter, in the bodies of the future ganglionic cells, there appears a clearly pronounced argentophyllic fibrillarity. Their peripheral outgrowths, penetrating the rudimentary receptor epithelium, branch at their ends and form nerve endings at the bases of the receptor cells. At the same time, the beginning of formation of a cuticle is visible at the apical ends of the receptor cells and sensory hairs may be seen on some cells.

At the end of the third day after hatching, the inner ear of the sturgeon is completely formed from the anatomical standpoint. A slight histological differentiation of the auditory maculae and cristae does continue. Sensory hairs develop on the surface of the receptor cells. Thus, when the sturgeon fry begin to breathe with gills, their ears are completely formed from the anatomical standpoint, and their auditory maculae and cristae have all of the most necessary elements for some kind of primitive functioning, i.e., their receptor epithelium already contains areas in which the receptor cells possess a cuticle and sensory hairs, while their basal portions are already in contact with the growing nerve fibers. The basic difference between these and the definitive maculae and cristae is that here not all of the receptor epithelium is differenti-

ated but only its central parts, while in the peripheral areas of the receptor epithelium there are still indifferent undifferentiated cells which may continue to multiply.

After gill breathing has begun, the most characteristic changes are observed in the ganglion of the eighth nerve (4 days after hatching). It consists of cells of two types: close to the macula there are cells with uniform, round, bright nuclei, containing 1 or 2 nucleoli and marked by very fine grains of chromatin; no particular polarization is apparent in them. In the central portion of the ganglion, the cells have nuclei of an irregular round or irregular oval shape. The nucleoli in them are hard to see. These cells have a clearly pronounced polarization, expressed primarily in the asymmetric location of the nucleus in the ventral-caudal portion of the cell. A clearly marked fibrillarity is visible within the cytoplasm. The nuclear chromatin is collected into large, closely packed lumps. In the peripheral portion of the ganglion, located close to the brain, the nuclei in the cells have a more elongated, oval shape and large, dark-colored lumps of chromatin. Mitoses are found in this part of the ganglion. Grains of pigment are scattered throughout the ganglion.

A second interesting feature is the increase in the concentration of thiol groups in both the receptor epithelium and the rudimentary ganglion. In the receptor epithelium, the maximum concentration of thiol groups is found in the cytoplasm of the receptor and supporting cells. Their concentration is somewhat lower in some of the receptor cells. The concentration of thiol groups is greater in the area of the cuticle and the basilar membrane. There are no thiol groups in the nuclear karyoplasm. The lowest concentration of thiol groups is found in the chromatin of the receptor and supporting cells. The shadows of the otoliths are visible beneath the macula, with a very weak concentration of thiol groups. In the ganglion, the distribution of thiol groups is higher in comparison with previous stages, but it is uniform. /85

On the 6th day after hatching, a sort of osmiophilic granularity appears in the mass of receptor cells, and this may be mitochondria. The mass of receptor cells takes on a characteristic pear shape. The mass of supporting cells, which extends from the basilar membrane to the apical surface, is clearly visible, especially when analyzing for carboxyl groups. The nuclei are located at different levels in the cell bodies, creating an impression of multistriation. The supernuclear portions of the supporting cells, considerably narrowed, pass between the bodies of the receptor cells and then broaden out again near the apical surface itself, located between the constricted apical portions of the receptor cells. All of the receptor cells exhibit sensory hairs whose tips rest against the otolith membrane. The nuclei of the receptor cells are round, with a definite nuclear membrane. The nucleoli are clearly visible. The chromatin in them is finely divided. The basal portions of the receptor cells are round, and appear in preparations stained with

silver and osmium as small baskets of nerve fibers, which are developed to different degrees in the individual receptor cells. One can often see cases where a nerve fiber has branched in two, forming synapses with two adjacent receptor cells. The bases of the receptor cells are located on a single plane, slightly above the middle of the receptor layer. The basilar membrane is well developed.

The content of nucleic acids shows no sharp changes at this stage. Their concentration is reduced somewhat in the basal portions of the cytoplasm of the supporting cells and increased in the apical portions of the receptor epithelium, mainly in the receptor cells. A high concentration of nucleic acids is observed in the nucleoli. DNA is found at the periphery (and RNA at the center) of nucleoli of receptor cells in preparations treated with methyl green-pyronine. At the same time, the receptor epithelium is strongly saturated with protein. Its concentration is particularly high in the enlarged apical ends of the supporting cells, while in the receptor cells it increases as one moves upward through the cell. The hairs of the receptor cells are poorer in protein than the cytoplasm. In the nuclei of receptor cells, the protein is concentrated mainly in the nucleoli. Its concentration in the chromatin is low. The basilar membrane is very rich in protein (Fig. 3).

The cells of the eighth ganglion are likewise rich in protein. Its concentration in the chromatin is minimal, while in other parts of the nerve cells (including the nerve fibers) it is close to maximal.

The carboxyl groups of the protein molecules give an interesting and highly differentiated picture. Their general concentration is somewhat less than the concentration of general or total protein in the same structures, but it is located in a greater variety of places. Strange as it seems, the highest (in fact, the maximum) concentration of carboxyl groups is observed in the otoliths. The /86 concentration in the receptor epithelium of the macula is much lower, however. If we consider only the receptor layer, the concentration of carboxyl groups will be found to be highest in the cuticular disks and in the apical, expanded portions of the supporting cells. On the other hand, the lowest concentration is found in the basal portions of the cytoplasm of supporting cells and in the chromatin. The concentration of carboxyl groups in the cytoplasm of receptor cells and in their nucleoli is medium, and lowest in the chromatin of their nuclei. A very low concentration of carboxyl groups can be found in the basilar membrane.

In the ganglionic cells, the concentration of carboxyl groups is generally the same as in the cytoplasm of the receptor cells. An increased concentration is found in some perinuclear portions of the cytoplasm and in the peripheral zone of the nucleolus.

The opposite picture is presented by the distribution of thiol groups. The entire receptor layer has a more or less uniform

concentration of them, which is the same in degree as that which was described in the previous stage. Against the general uniform background of the receptor layer, only the nuclei are visible, due to their completely colorless karyoplasm. Here again the nucleoli are indistinguishable from the general background. In the ganglion, there is the same uniform picture of distribution of thiol groups, only their concentration here is somewhat higher than in the previous stage. In this case, however, the nucleoli of the ganglionic cells stand out against the general uniform background owing to their higher concentration.

On the 8th day after hatching, the external appearance of the epithelium is approaching the definitive form. The number of receptor cells has increased owing to the differentiation of the peripheral areas, and they all have a characteristic pear shape. A well-developed cuticle and sensitive hairs are to be seen at their apical ends. Nerve fibers, which entwine the base of each cell and are strongly impregnated with silver, run to their basal portions. A linear osmiophyllic fibrillarity is visible in the bodies of the supporting cells which run to the apical surface of the epithelium; they evidently form a system of supporting fibrils. Above the sensory hairs is the otolith membrane, with small groups of otoliths distributed over it more or less uniformly.

The subjacent ganglion of the eighth nerve has a more differentiated appearance. The nerve cells are somewhat larger in size, mainly at the expense of the cytoplasmic body of the cell. The bright oval nucleus, very poor in chromatin, is located asymmetrically, close to that pole of the cell from which the peripheral process extends. A cartilaginous capsule is formed around the internal ear.

Hence, the inner ear of the beluga appears to be completely formed in all important respects (both anatomically and histologically) by the time the fish begins to feed.

At this stage, the nucleic acids are to be found in more specific locations. The cytoplasm contains a smaller concentration of RNA in the basal portions of the supporting cells, which increases in the apical zone of the receptor epithelium. An increase in the concentration of RNA in the apical portions is especially marked in the cytoplasm of the receptor cells. The nuclei of the receptor cells are large, slightly oval in shape, and arranged in one or two rows; the DNA concentration in their chromatin is generally the same as that of the RNA in their cytoplasm. A significantly higher concentration of nucleic acids is found in the nucleoli of the receptor cells, which have a mixed appearance: the centers of these nucleoli, as we have already said, contain RNA, while DNA is found in the periphery. The latter is clearly visible in the reaction with methylgreen pyronine and in the Felgen reaction.

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The picture for the distribution of the general or total protein

is the same as in the preceding stage. It is observed in both the receptor epithelium of the macula and in the cristae. The receptor cells of the maculae and cristae at this stage differ mainly in the size of the sensory hairs, which are much longer in the cristae than in the maculae.

A very clear and sharp picture of the cytochemical structure of the macula is given by the carboxyl group of protein molecules (Fig. 4). The increase in their concentration is more clearly pronounced in the apical portions of the receptor cells. As before, the maximum concentration is found in the otoliths. The chromatin, nucleolus and cytoplasm of the receptor cells generally have a singularly high concentration of carboxyl groups. Once again, a lower concentration of carboxyl groups is found in the supporting elements and in the basilar membrane.

The localization of carboxyl groups in the ganglion generally remains the same. The only new feature is the presence of ganglionic cells with different degrees of concentration of carboxyl groups.

After active feeding has begun, the inner ear of the beluga shows signs of activation of processes of formation of the otolith membrane in both the saccule and utricle. Individual otoliths, with a pronounced crystalline hexahedral form, are visible against a background of bright, unstructured substance, along with oval-shaped otoliths having rounded corners. Fibrous structures of some type then appear in the otolith membrane itself. It is difficult to say whether they are found in the living ear or whether they result from the fixing process; it is definite, though, that their appearance has to do with some substantial changes in the substance of the otolith membrane.

In the receptor epithelium of the utricular and other maculae, after active feeding has begun, one can detect a denser arrangement of receptor cells and the combination of individual receptor cells into groups, linked with a common innervation by a single nerve fiber. The formation of such groups is most clearly evident in the central portions of the maculae and cristae.

Different portions of the ganglion are in various stages of differentiation. Thus, the peripheral portions of the ganglion and its caudal portion are composed mainly of fine cells with scarcely visible cytoplasm. In the cranial portion, on the other hand, there are larger ganglionic cells which are closer to the definitive ganglionic cells in their structure and form.

Well-developed auditory ridges (cristae) are visible in the ampullae. Their receptor epithelium shows differentiated receptor cells with nerve fibers running to them; the latter are clearly visible in preparations impregnated with silver. A cuticle is located on the surface of the cells. Long sensory hairs proceeding from the receptor cells penetrate the surface of the cupola located above

them; in fixed preparations, the latter has a fibrous structure. We were unsuccessful in following the development of the cupola at all stages of embryogenesis, since it is very poorly preserved in preparations fixed and immersed in paraffin, and we did not have any material available in this case for *in vivo* observations. For this reason, we are omitting any description of the development of the cupola in ganoid fishes.

At these stages, in preparations impregnated with silver, the penetration of the central growths of the ganglionic cells of the eighth nerve into the brain and their endings in the cells of the Deiter's nucleus are clearly visible.

For the period from the 12th to the 21st day (when we concluded /88 our observations), the processes of differentiation in the ganglion of the eighth nerve were most characteristic. The inner ear and brain are covered by a common cartilaginous capsule. Differentiation of the ganglion is accompanied by processes of complication of the innervation of the receptor epithelium in the maculae and cristae. Obviously, the differentiation of the receptor epithelium is largely complete at the beginning of active feeding, during which the larva must possess a sufficient degree of motility and coordination of motion while in pursuit of its prey. Further complication of the inner ear involves mainly the development of the synapses, further development and more complex interweaving of the innervating epithelium of the nerve fibers, and the strengthening and development of the links with the brain. Development of the otolith membranes continues in the maculae. The process of the combination of individual small otoliths (otoconia) into one large mass in a portion of each ampulla, described by several authors (de Burlet, 1934), was not seen in our material. Evidently this process occurs at later stages.

The distribution of nucleic acids generally changes little after active feeding has commenced, except that their concentration decreases in individual receptor cells; this evidently is related in some manner to an increase in their specific function. The basilar membrane appears very dark when stained with galloxyanin; this is somewhat difficult to explain, since such a color is indicative of a high content of nucleic acids. The impression is created that the basally-located nuclei of the supporting cells have become somewhat finer and have moved closer together. However, such a conclusion requires statistically reliable qualitative data, which we are lacking.

The nuclei in the ganglionic cells are poor in DNA. The nucleolus is distinguished by a slightly higher concentration of nucleic acids, mainly at the expense of RNA. Cell-satellites stand out exceptionally clearly and constitute a higher concentration of nucleic acids than do the ganglionic cells.

The highest concentration of protein is found in the cuticle and in the external plasmatic membranes of the receptor cells. In

the cytoplasm and nucleus of the receptor cells themselves, the concentration of total protein is higher than in the supporting cells. The sensory hairs of the receptor cells are still poorer in protein than is their cytoplasm.

The ganglionic cells, as far as their chromatin and nucleoli are concerned, resemble the supporting cells in protein content. Among the cell-satellites there are some whose nuclei are very poor in protein, while their cytoplasm is very rich in it.

In general, the carboxyl groups also retain the characteristics of their distribution at much earlier stages. Vacuolization occurs only in the basal portions of the receptor epithelium (Fig. 5).

The concentration of thiol groups in the receptor epithelium decreases, being retained only in the apical zone. It is practically nonexistent in the sensory hairs. The cytoplasm of the receptor cells of the macula appears vacuolized in the basal portions and denser in the apical portions. In general, the distribution of thiol groups in the receptor epithelium has a more diffuse character.

Hence, we succeeded in following the development of the inner ear of ganoid fishes, described here with the example of the beluga, using both general histological and cytochemical methods, up to the 21st day after hatching. The beginning of active feeding is apparently the start of normal functioning of all receptor structures of the inner ear, and further cytochemical processes depend not on processes of differentiation of receptor structures, but on their function. Complication of the functioning of the vestibular apparatus during further development of the larva is evidently dependent upon the development of its nerve apparatus and the maturation of the central areas of this analyzer. The task which we set ourselves did not include study of these processes. /89

Unfortunately, we did not have the possibility of conducting histochemical and morphological studies of the inner ear of adult ganoid fishes, let alone carrying out a functional investigation of them.

The embryonal development of the inner ear was studied simultaneously in the sturgeon and sevruga as well as the beluga. All of the regular features mentioned above apply to these two forms as well. We shall not dwell on a detailed description of them, since the basic steps of morphological and cytochemical histogenesis are exactly the same in these fishes.

In the embryonal development of the inner ear of ganoid fishes, there is very clear evidence of the link between the change in distribution and degree of intensity of the reaction to nucleic acids, total or general proteins, and functional groups of protein molecules, and the character of the processes taking place in the developing receptor formations being studied and in their cellular elements.

In the rudimentary receptor epithelium, as in the rudimentary ganglion, in the early stages prior to the beginning of differentiation (indifferent stage), a rather high reaction intensity is observed with regard to all of the biologically active substances mentioned above. Characteristic of this stage is their uniform distribution both within the limits of the entire rudiment and in individual components of its cellular elements. The first sign of the beginning process of differentiation (formation of the macula communis) is an increase in the nucleic acid concentration and a rise in the amount of protein and its functional groups in the nucleolus, accompanied by an increase in the dimensions and a shift of the nucleolus to one of the poles of the nucleus. Continuation of the process then leads to a slight fall in the concentration of the above-mentioned substances, indicated by a decrease in the intensity of the reaction in the cytoplasm of the cells mainly with respect to RNA, then to protein, and sometimes also to the carboxyl groups of the protein molecules.

During the division of the macula communis into the rudiments of the individual receptor organs, which is usually linked with an increase in the mitotic activity in the rudiments of the sensory epithelium, a process of accumulation of the above substances can be seen, linked with an increase in the intensity of the reaction and usually unaccompanied by a redistribution of these substances within the rudiment. In return, therefore, the processes of its cytological differentiation are always linked firstly with some source of RNA and protein and secondly with their redistribution, the nature of which is determined by the cytochemical characteristics of the definitive receptor and supporting cells in animals of a given type. A typical example of such a "redistribution" is the drop in the concentration of DNA in the chromatin of the nuclei of the receptor cells, linked with an increase in their size, as well as their increase in the nuclei of the supporting cells, whose size thereupon sometimes decreases. The simultaneous rise in RNA concentration which is observed in the apical portions of the cytoplasm of the receptor and supporting cells is evidently linked to further formative processes at their summits, i.e., with the formation of tufts of sensory hairs and a cuticle in the receptor cells and desmosis in the vicinity of the summits of the supporting cells. The change in the RNA distribution, therefore, is undoubtedly linked with the differentiation processes and is apparently functional in this regard.

The process of differentiation of the maculae and cristae in ganoid fishes does not occur simultaneously within the entire rudiment, but begins at the center of the receptor formation and gradually expands toward its periphery.

The cytological differentiation of the receptor formations of the inner ear of ganoid fishes characteristically involves the active participation in this process of the carboxyl groups of protein molecules and the absence of visible changes with respect to the content and localization of thiol groups. We were unfortunately unable

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to study the cytochemical features of later stages of differentiation of the receptor cells of the inner ear in ganoid fishes, nor did we find any information on this subject in the literature available to us.

In the differentiated receptor cells of the inner ear of the larva in ganoid fishes, there is evidence of a high concentration of RNA and protein as well as a simultaneous decrease in the concentration of carboxyl groups of protein molecules. However, unlike the remaining undifferentiated cells, they gradually shift (concentrate) in certain cell structures. A characteristic feature of thiol groups in the receptor formations of the inner ear of ganoid fishes is the uniformity of their distribution both in the elements of the receptor epithelium and in the neurons of the ganglion of the eighth nerve, in which their concentration is slightly higher.

The concluding stages of differentiation of the receptor epithelium of the inner ear are marked by a characteristically denser arrangement of receptor cells, the final differentiation of the entire receptor formation as a whole, and the combination of individual cylindrical receptor cells into groups linked by a common innervation. The separation of such groups is most easily followed in the central portions of the maculae and cristae.

The final stage of differentiation is also linked to the conclusion of differentiation of all ganglionic cells in the entire ganglion of the eighth nerve, at the same time that there is a complication of the innervation of the sensory epithelium of the maculae and cristae. The latter process is connected with the development of the synapses, an increase in the dimensions of the plexus externus and the ramification of the network of nerve fibers in it, as well as the development and growth of the links to the brain.

DEVELOPMENT OF THE INNER EAR IN TELEOST FISHES

The development of the inner ear in teleost fishes (and in the groundling in particular) follows essentially the same pattern as that for ganoid fishes, differing from it in only a few respects.

The primary rudiment of the inner ear, the dual auditory fossa, is observed in the neurula stage on both sides of the nerve ridges, while at the stage of early caudal development there is a separation of the indifferent auditory vesicle. It increases in size both by cell division and by stretching of the walls, apparently as a result of accumulation of fluid within the vesicle. One of the walls becomes thicker to form the so-called macula communis, the single rudiment of all future receptor structures of the inner ear. At the same time that the auditory vesicle is growing, the macula communis is also increasing in size. Almost simultaneously with the formation of the latter, the rudiment of the ganglion of the eighth nerve appears, having an apparently mixed origin (from the wall of the auditory vesicle and from the ganglionic lamina). We did not succeed

in demonstrating this fact conclusively in the groundling, however. At the end of embryonal development (4th stage of the egg phase, according to Rass), there is a division of the auditory vesicle into two parts: the so-called pars superior, from which the utriculus and the ampullae of the anterior semicircular canals subsequently develop, and the pars inferior, from which the saccula, ampulla of the posterior semicircular canal, and lagena are formed. At the same time that the auditory vesicle divides, the macula communis also splits into two parts, from which the utricular macula later develops, initiating formation of the otolith. Hence, prior to the hatching of the larva from the egg, its inner ear already contains the rudiments of two receptor formations and an otolith above one of them. /91

The development of the inner ear of teleost fishes differs in one respect which apparently reflects the phylogenetic features of the inner ear of vertebrates: the development of the ampullae and the differentiation of the cristae do not occur simultaneously. As was pointed out previously by earlier authors (Kolmer, 1927) and confirmed by us in the groundling, the anterior vertical semicircular canal develops first, followed by the posterior vertical and then the horizontal semicircular canals. However, the rudiments of the cristae of these canals in the groundling deviate somewhat from this order: the cristae of the two anterior semicircular canals are formed as a common rudiment, which then splits into two cristae. All of this differentiation does not take place simultaneously, however; first differentiation begins in the crista of the vertical canal, and somewhat later in the crista of the horizontal canal.

The histochemical studies which we conducted made it possible to show the staggered morphological and cytochemical differentiation of all three maculae: differentiation begins in the macula utricularis, then occurs in the macula saccularis and finally in the macula lagenaris. This course for the process coincides with the development of the inner ear in the course of phylogenesis.

After hatching, during the larval stage, anatomical development of the inner ear and differentiation of the macula utricularis, as well as development of the otolith, take place. Differentiation of the macula saccularis and the cristae occurs mainly at the passively drifting stage of the larva. The beginning of active feeding, connected with the active motility of the larva, is characterized mainly by considerable progress in the differentiation of the brain at the level of Deiter's nucleus, the presence of differentiated areas in the macula utricularis and macula saccularis, the early stages of differentiation of the third macula (macula lagenaris), and the presence of differentiated areas in the cristae. Differentiation of the receptor layer of molecules and cristae generally does not occur simultaneously throughout the rudiment, but from the center toward the periphery, so that all during the embryonal development of the inner ear, in each of its receptor formations, one can find both differentiated central and less or completely

undifferentiated peripheral areas, which show up especially clearly in studies of the proteins and nucleic acids. Twenty hours after hatching, the inner ear of the groundling is completely differentiated, but its growth and the growth of the otoliths continue throughout the life of the individual fish.

As far as its structure is concerned, the auditory vesicle is composed of large undifferentiated cells, initially connected to the adjacent ectoderm (Fig. 6). The cells in the wall of such a vesicle are arranged in a single layer, and have rather large oval or circular nuclei, each containing 1 or 2 large nucleoli. The cytoplasm of these cells contains a moderate amount of RNA granules, producing a reaction of average intensity. Their nucleoli are stained very brightly, indicating a high concentration of both DNA and RNA in them. The cells of the adjacent ganglion are marked by a much more intense reaction to both RNA and DNA. The intensity of the reaction to total protein at this stage is also low, increasing somewhat toward the apical parts of the cells. The reactions to carboxyl and especially thiol groups are still milder.

In the ganglionic cells, the reaction to protein and its functional groups is not uniform: it is weaker in some cells and stronger in others, but generally does not exceed average intensity. At the beginning of formation of the common rudiment of the receptor epithelium, the so-called macula communis, there is a general drop in the intensity of the reaction to RNA, total protein and carboxyl groups. The intensity of the reaction in individual cell elements is uniform, however. /92

Prior to division of the macula communis into two parts, it shows an increase in the intensity of the reaction to DNA, RNA, total protein, and carboxyl groups (Fig. 7). The nuclei stand out more sharply and clearly in the Brach reaction for nucleic acids. Their karyoplasm shows up more darkly. In the protein reaction, there is a general increase in their intensity in both the nucleus and cytoplasm. The rudiment of the ganglion of the eighth nerve loses its uniformity, as is clearly evident when the macula communis divides. In its medio-caudal portion, the majority of cells assume a characteristic shape, but their cytoplasm is relatively low in volume and very rich in brightly stained RNA granules. The nuclei of these cells are rounded in shape, poor in chromatin, and contain nucleoli which are poor in RNA. The remaining cells of the rudiment of the ganglion are smaller in size, as are their nuclei. Their cytoplasm is poor in RNA. The nuclei are poor in DNA, but have very clear nucleoli rich in RNA. Prior to hatching, the rudiment of the inner ear shows an increase in RNA content, protein and its functional groups. The most intense protein reaction is seen in the nuclear membrane and the nucleolus. The developing otolith gives a mild reaction to total protein.

The processes of further morphological and cytochemical differentiation take place in all receptor structures of the inner ear,

usually at the same time but in some cases at different times.

After hatching, the larvae show formation of the macula utricularis and quite intense growth of its otolith. The epithelium of the macula is in two layers. The cytoplasm of its cells is very pyroninophilic, that is to say, the stain is much brighter than in earlier stages of development of the auditory vesicle. The nuclei are dark blue (when stained according to Brach) and turbid, so that their structure cannot be seen. The otolith lies above the middle portion of the macula and consists of two layers. At its center is a rounded, colorless "nucleus", separated by a thin dark line from the outer yellowish-grey layer. Staining of the otolith is nonspecific; it has an irregular rounded shape, but its outline (where it touches the macula) is very even and flat. When a section of it is tested for total protein and carboxyl groups, the otolith gives a weak yet definitely positive reaction. The reaction in the rudiments of the sensitive epithelium is somewhat more intense, while that in the epithelium lining the wall of the ear is most intense. The macula is seen to contain receptor cells which differ from the supporting cells in their high protein content. In the nuclei of the receptor cells, on the other hand, the nucleoli show up very clearly. The common rudiment of the cristae of the anterior ampullae is divided into two rudimentary structures.

In the adjacent ganglion, the cells are distinguished by a low protein content and the presence of a nucleolus; the latter is rich in protein, however. The cytoplasm is much darker than the nuclei. It is easy to trace the nerve fibers running from the ganglionic cells to the brain, but they give the impression of not being fully differentiated at this stage. It is interesting to note that at this stage the intensity of the reaction for carboxyl groups in the receptor epithelium is much weaker than the intensity of the reaction for total protein. It is somewhat higher in the ganglion, attacking average levels, but differs in individual cellular elements. /93 At the same time, mention should be made of the fact that processing for carboxyl groups shows up in extreme clarity the features of the fine structure of the various cells and tissues. In this reaction, the otolith shows up as very pale and completely uniform. The ganglion is surrounded by brown pigment, which also appears around the developing inner ear. In the reaction for thiol groups, the brightest areas are represented by the nucleoli, in which the concentration of thiol groups attains average intensity. The fine structure of the macula cannot be distinguished in this reaction, since the concentration of thiol groups is more or less uniform in both the nucleus and cytoplasm. The karyoplasm of the nuclei is also stained, but more brightly.

When active feeding begins, the most characteristic feature is the highly advanced differentiation of the brain (at the level of Deiter's nucleus), indicating establishment of the link between the receptor structures of the inner ear and the central nervous system, reinforced by the presence of synapses between the fibers of the

eighth nerve and the large neurons of the brain. In sections, the ear appears to be rather complexly organized, although a definitive configuration has not been attained. At the center of the macula utricularis, beneath the otolith, one can see the well-differentiated cylindrical sensory cells and the supporting cells accompanying them. Due to the low protein concentration, the sensory hairs can be seen only with difficulty. Along the edges of the macula there are still undifferentiated cells, marked by a high RNA content relative to the differentiated cells as well as a lower protein content (Fig. 8).

The processes of cellular differentiation continue in the subjacent ganglion. The individual ganglionic cells come to resemble the differentiated cells in their characteristics, but differ from them in a lack of tigroid and the presence of an eccentrically located nucleolus rich in RNA and protein. Thiol groups are most poorly represented in the cells of the inner ear. Their concentration is somewhat greater in the subjacent ganglion, where they are richest in the perinucleonem of the nucleolus, the nuclear membrane, and the cytoplasm of more differentiated cells. It is immediately apparent that the thiol groups are arranged in different ways in the ganglion, while their arrangement in the receptor structures of the inner ear is very uniform.

Two days after the beginning of active feeding, the maculae and cristae show a gradual increase in the number of differentiated receptor cells, with accumulation of granular RNA in the apical and basal portions of the sensory cells. The supporting cells are much poorer in RNA. The appearance of Nissl's substance can be seen in individual ganglionic cells. In preparations treated for total protein, a sharp increase in the concentration of the latter is observed in the sensory epithelium and especially in the ganglion of the eighth nerve. The sensory epithelium of the macula is dark, becoming somewhat more pale at the apical ends. The sensory hairs are colorless as usual. The receptor epithelium of the crista has the same appearance, but here the protein content is more uniform over the entire length of the receptor layer.

Four days after the beginning of active feeding, all three maculae appear to be completely differentiated, but their cytochemical differentiation and the degree of functioning apparently differ somewhat. In preparations treated for nucleic acids according to Brach, all three maculae differ from one another in the content and distribution of RNA. The macula saccularis is poorest in RNA. As usual, the cristae appear somewhat less differentiated, but nothing can be said about the development of the cupola. In the ganglion /94 of the eighth nerve, the majority of ganglionic cells increase considerably in size as their cytoplasm increases; the latter is markedly rich in RNA. The RNA distribution in them changes in connection with the formation of Nissl's substance (Fig. 9). The development of the ear has become anatomically definitive, varying from this state, perhaps, in the purely dimensional relationships of the indi-

vidual parts. For the first time, the sensory hairs become highly visible in the protein reaction, although the protein content in them is very small and they appear nearly colorless in both the reaction for total protein and that for carboxyl groups. In the reaction for thiol groups, the presence of a light staining can be discerned in them. In all protein reactions in the receptor layer, the apical ends of the sensory cells and their cuticle show up darkest. The content of thiol groups is the same in both the cytoplasm and the karyoplasm of the receptor cells; a high concentration can be seen in the nuclear membranes and chromatin. The receptor cells of the macula utricularis often show a different degree of eccentric location of the nucleolus, as well as its emergence into the basal portion of the cell; the latter event is especially pronounced in the reaction for carboxyl groups. This process is evidently linked to the incipient function of the macula utricularis. In the ganglionic cells, the most definite reaction for total protein is observed in the perinucleonem of the nucleoli. At this stage, the cytochemical differentiation of the cellular elements of the auditory spots or maculae (macula utricularis, macula saccularis, and macula laminaris) is evidently complete, and the slight shifts of protein and RNA that can sometimes be seen in them are basically linked to the specific function of those receptors. However, the process of differentiation continues for some time in the cristae. Until the 19th day, there is a definite cytochemical difference between the central and peripheral parts of the crista, which are represented at this stage by still incompletely differentiated cellular elements. It is interesting that the content of nucleic acids in them is higher (but the total protein is lower) than in the differentiated central portions. The differentiation of the cristae is complete by the 20th day, when the cytochemical difference between the central and peripheral portions of the cristae disappears.

A study of the glycogen has shown that in the groundling, at the stage of early caudal development, all the cells of the auditory vesicle are filled with glycogen, as are all the other tissues of the embryo. The glycogen granules are packed so densely that not even the nuclei can be distinguished within the cells. As development proceeds, the amount of glycogen in the cells decreases somewhat, so that by the stage at which the head separates it is possible to see easily the colorless nuclei in the cells of the auditory vesicle. When the young hatch from the egg (4th egg stage according to Rass, 1925), glycogen is completely absent from the rudiment of the inner ear. A pink coloration is visible in the apical ends of the cells in the rudiment of the receptor epithelium and the cell walls, but here the positive Schick reaction is not related to the presence of glycogen; it apparently depends on the presence of mucopolysaccharides. Glycogen is found in very small amounts in the form of clear granules in the basal portions of some cells. The cells in the rudimentary ganglion are free of glycogen. The nerve fibers give a mildly positive Schick reaction, which is in all probability linked to the presence of some mucopolysaccharides in them.

After hatching (6 days after fertilization), glycogen is completely absent from the rudiments of the receptor epithelium of the maculae. A mildly positive Schick reaction, not related to glycogen, is given by the intercellular boundaries, while a very definite reaction is obtained in the basilar membrane and the otoliths. At this stage, glycogen is found only in small amounts in the cells of the rudiment of the ganglion of the eighth nerve. On the 7th day, /95 there is also a mildly positive reaction in the rudiment of the otolith membrane.

The macula utricularis of the passively drifting larva has a light pink background and somewhat larger granular inclusions of glycogen in the apical and basal portions of the cells. The sensory hairs are colorless. The cells with glycogen inclusions are located at the central portion of the macula beneath the otolith, which gives a very definitely positive Schick reaction. The latter is not removed by saliva, however; i.e., it does not depend on the presence of glycogen but on some mucopolysaccharide. The otolith has a stratified appearance: at the center there is a paler "nucleus", and around it are two concentric layers, the inner one darker and the outer one lighter. We have been unable to determine the nature of this Schick-positive substance (Fig. 10).

There is no glycogen in the ganglion of the eighth nerve at this stage.

In the actively moving larva, after the beginning of active feeding (14 days after fertilization), large and very clear granules of glycogen are visible in all receptor cells of the macula in their basal portions (Fig. 11). The sensory hairs are pale pink in color. All of the remaining cells of the receptor epithelium give a mildly positive Schick reaction, which is not destroyed by saliva. A quite definite Schick reaction is given by the nerve fibers which terminate at the sensory cells; a control treatment with saliva shows that in this case glycogen is present. As before, the otolith gives a very definite Schick-positive reaction. After fixing with Shabadash neutral fixer, the stratifications mentioned earlier stand out more clearly. The Schick-positive reaction in the otolith increases significantly in the larva following the beginning of active feeding. At this stage the glycogen also appears in the receptor cells of the cristae.

In the cytoplasm of the cells of the ganglion of the eighth nerve, there is a light pink color which is removed by saliva.

In actively feeding larvae of more advanced age (16 days after fertilization), the amount of glycogen in the receptor cells of the maculae and cristae increases; this is evidently linked to the continuing differentiation and the increase in the number of differentiated receptor cells in these formations. A definitely positive Schick reaction is produced by the apical surface of the receptor epithelium; it is evidently connected with the developing cuticle.

This coloration is not removed by saliva, however. The pink color is retained in the ganglionic cells, but there are no granular inclusions of glycogen in them. The otolith retains the former Schick-positive reaction. The same pink color is shown by the limits of the cells, which retain it even after the control treatment with saliva. The hairs have a pink color after fixing in a saturated solution of picric acid in absolute alcohol, and appear colorless after fixing with a neutral Shabadash mixture. The same picture is seen in larvae of more advanced age.

Summing up briefly what we have stated above, we can say that the organotypical period in the development of the inner ear of the groundling is already complete at the protolarval stage, but does not occur simultaneously for all receptor structures. The period of cytological differentiation comes somewhat sooner in the macula utricularis than in the macula saccularis and the macula lagenaris; in the cristae, it starts in the vertical canals and then in the horizontal ones. The end of differentiation is observed in the same order. This step-by-step development of individual receptor structures reflects the path of phylogenetic development of the inner ear and shows up more clearly in the ontogenesis of lower vertebrates, tending to disappear in higher ones. Accumulation of RNA and protein always precedes the start of cytological differentiation, but its course is usually linked with a drop in the concentration of RNA and protein in the cytoplasm of differentiating cells and a shift of the nucleolus toward the basal pole of the nucleus, i.e., toward the nuclear membrane. Differentiation of the ganglionic elements of the ganglion of the eighth nerve takes place parallel with the receptor structures of the inner ear, the differentiation of whose individual parts also differs somewhat in time. The cytological differentiation of all receptor structures of the inner ear follows the exact same pattern: at the start, a common cellular rudiment divides into the supporting and future receptor cells, after which the fine processes of differentiation begin within these cell groups. It does not occur simultaneously all through the rudiment, but begins in its central portions and moves toward the periphery. In the macula, this process is linked to a significant degree with the development of the otolith, the establishment of a connection with the differentiating central nervous system, and with the effect of the external medium on the developing organ. Morphological aspects of differentiation include changes in the shapes of the cells, as well as the formation of synapses, cuticles, and sensory hairs on the receptor cells; cytochemical changes include a change in the concentration of protein and nucleic acids, and their redistribution within the cell itself.

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The study of the development of the inner ear of the groundling has shown very marked time differences for the periods of the beginning of differentiation of various receptor formations of the inner ear, with uniformity of the actual process of organic and cellular differentiation. One might think that these time differences were linked with a reflection of the phylogenetic development of this

organ in its ontogenesis. This is especially true of the difference in the times of beginning of differentiation of the maculae and cristae on the one hand and the development of the cristae of the anterior vertical and horizontal semicircular canals from a single rudiment on the other. This can be understood from the standpoint of the later appearance of the horizontal canal in phylogenesis. It is more difficult to explain the reason for the difference in time for the beginning of differentiation of the macula utricularis, macula sacularis, and macula lagenaris, since we know practically nothing about their phylogenesis within the limits of development of the vertebrates.

A characteristic feature of the undifferentiated auditory vesicle of the groundling is the average concentration of nucleic acids and protein, the somewhat smaller amount of carboxyl groups and the small quantity of thiol groups of protein molecules. However, the concentration of nucleic acids and protein is very high in the nucleolus. A high intensity for these reactions is typical of the rudimentary ganglion, in contrast to the individual cells.

The concentration of these substances decreases upon formation of the common rudiment of the receptor organs of the inner ear, the so-called macula communis. The period of time between its formation and its division into separate rudiments is characterized by the accumulation of these chemical, biologically active substances, expressed histochemically by an increase in the concentration of these substances. When the epithelial cells are modified to become receptor and supporting cells, a rather high concentration of RNA is retained in the cytoplasm of both varieties. The cytochemical differences lie mainly in the DNA concentration in their chromatin. It is interesting that the protein has a higher concentration in the receptor cells than in the supporting cells, in which a very high concentration of protein is found only in the nucleolus. The concentration of carboxyl groups in the receptor epithelium decreases, but at the same time it becomes very easy to distinguish the structure of the individual cells and their components. The thiol groups give a weak and very uniform reaction throughout the whole rudiment, which becomes more intense in the nucleolus. /97

The differences in the concentration of protein in the differentiated and undifferentiated cells can be seen clearly in various receptor organs of the inner ear of the larval groundling following the start of active feeding, when each receptor structure contains central differentiated and peripheral undifferentiated parts. There is an inverse relationship between the reactions to nucleic acids and proteins; while the cells of the undifferentiated parts are marked by a much higher content of RNA relative to the differentiated parts, they have a characteristically smaller concentration of total protein.

The rudiment of the receptor epithelium is poor in thiol groups, which are evidently only remotely connected with the processes of

cellular differentiation. In the differentiating ganglion, however, the concentration of thiol groups is much higher than in the receptor epithelium, and their distribution during the differentiation of the ganglionic cells changes noticeably, which is indicative of their direct involvement in the differentiation of the ganglionic cells.

Subsequent processes of cellular differentiation are linked to a considerable degree with the redistribution of RNA and a change in its concentration. Thus, the differentiating receptor cells show an accumulation of granular RNA in the apical and basal parts of their cytoplasm, while there is a simultaneous lack of RNA in the cytoplasm of the supporting cells. The final stages of differentiation of the ganglionic cells are linked with the appearance in their cytoplasm of Nissl's substance, which is characteristic of a high RNA concentration. A characteristic feature of differentiated receptor cells is the increase in intensity of the protein reaction in the basal and perinuclear zones of the cytoplasm and in the supporting cells. There is very little of it in the sensory hairs. Their reaction to protein increases toward the end of differentiation.

As the cuticle develops, the intensity of the protein reactions increases in the apical portions of the receptor cells and in the cuticle.

A study of glycogen in the process of development of the inner ear of the groundling has shown that in the early stages of the rudiments of the auditory vesicle (until the stage of the separation of the head), all the tissues of the embryo (including the rudiment of the auditory vesicle) are filled with glycogen, the amount of which decreases sharply in the course of further embryonal development and completely vanishes from the rudiment of the internal ear when the larva hatches. In protolarvae, glycogen inclusions are completely absent from the receptor cells in the rudimentary tissue, and there is only a mildly positive Schick reaction in the cell membranes and nerve fibers. A sharply positive Schick reaction is given by the developing otolith, but this is related to mucopolysaccharides and not glycogen. Inclusions of glycogen appear secondarily in the centrally located receptor cells of the maculae and cristae in the passively drifting larva. The amount of glycogen and the number of receptor cells containing it increase sharply in the actively feeding larva.

THE DEVELOPMENT OF THE INNER EAR IN TAILLESS AMPHIBIANS

The study of the development of the receptor structures of the inner ear in tailless amphibians is of particular importance because the adult representatives of this order have come out on dry land and have completely changed both the position of the trunk relative to the surface of the ground and the means of locomotion, while their larvae have retained an aquatic form of life and a fishlike form.

At the stage of caudal development, the structure of the audi- /98

tory vesicle has an undifferentiated appearance. The auditory vesicle is completely closed and separate from the ectoderm, while its wall consists of large, highly vacuolized cells rich in yolk and having dark, homogeneous nuclei of irregular shape. In the latter, it is usually easy to see dark nucleoli, which are located eccentrically in some cells. The apical surface of the cells is uneven, and their cytoplasm in the apical portions is clogged with grains of brown pigment. There is no cuticle on the surface of the cells.

The dimensions of the auditory vesicle increase as the time of hatching approaches. A large part of the wall becomes thinner and the cells composing it assume a more regular arrangement (stage 18 according to Terent'yev). Their apical ends flatten out to form a more or less flat surface. The nuclei are dark and homogeneous. The nucleoli are clear and hard to see against the general background of the nucleus. The cytoplasm of the cells is highly vacuolized. Large grains of yolk remain in the cells. The apical ends of the cells contain grains of pigment, but they are somewhat less densely packed than at much earlier stages (Fig. 12). The auditory vesicle contains the rudiment of the endolymphatic diverticulum. The ganglionic rudiment adjacent to the auditory vesicle consists of densely packed neuroblasts. The nuclei are large, clear, and oval in shape. Large grains of yolk and granules of pigment can be seen between them. One of the walls of the auditory vesicle thickens and becomes multilayered. Obviously, this forms the basis of the macula communis. Unfortunately, its cytological structure is very difficult to make out, owing to the presence of a large number of coarse grains of yolk, which sometimes obscure the nuclei themselves. The pigment granules, which are also present in large numbers, have the same disturbing effect. Toward the end of this stage, the auditory vesicle divides into the pars superior and the pars inferior. Nerve fibers can be seen in small numbers in the ganglion.

Under laboratory conditions, the hatching of the larva from the egg does not occur at the same time for all fry. Some of them "fall out" of the shells at the 18th-19th stages, when their ears have developed only to the extent of dividing into two parts, so that those larvae which have "fallen out" lie on the bottom of the tank either completely immobile or reacting to stimuli with muscle contractions.

At the 20th stage, when the larvae normally emerge from the eggs (Terent'yev, 1950), the ear already has a complex configuration. It has both an utricle and saccule, as well as the semicircular canals. The rudiments of the receptor epithelium can be distinguished as consisting of multiple layers. The nuclei of the cells composing them are large and round. The reaction to DNA produces a diffuse coloration of the nucleus. The nucleolus is marked by a high concentration of DNA, but its pyroninophilicity is insignificant; from this we may conclude that RNA is present in the nucleolus in a negligible concentration. The concentration of RNA in the cytoplasm is low and very much obscured by the granules of pigment. We did not succeed in observing the division into receptor and supporting cells.

The RNA and DNA concentrations are weaker in the subjacent rudimentary ganglion. In individual rudiments, however, cells are found with a high RNA concentration in the cytoplasm.

During the 21-22 stages (according to Terent'yev), when the tadpole is able to open its mouth and the cornea of the eye becomes transparent, formation of maculae and cristae takes place in the inner ear. The concentration of DNA increases in the nuclei of the receptor epithelium. Clear nucleoli stand out against a background of colorless karyoplasm. The limits of the cells are not visible. The receptor epithelium contains many grains of pigment, which conceal the cytoplasm. The apical portions of the cytoplasm remain free of pigment, and a high concentration of RNA can be found in them. In comparing the individual parts, we can assume that the RNA concentration is weaker in the basal portions of the epithelial layer and increases in its apical portion. The abundance of pigment grains makes it difficult to obtain a full and reliable picture. /99

In the macula, as in the crista, the entire thickness of the epithelium is filled with nuclei, arranged in several rows. The cytoplasm is visible only in the apical and basal portions. In preparations treated for proteins and for carboxyl groups of protein molecules, it is impossible to follow the contours of the cells. The entire mass of nuclei appears uniform. The contours of the nuclei are very distinct and dark. Their chromatin is characterized by a low concentration of protein and carboxyl groups. Against the background of bright chromatin and colorless karyoplasm, the dark nucleolus stands out sharply, marked by a higher concentration of protein and carboxyl groups of protein molecules; the degree of their concentration in the cytoplasm approaches that in the nucleolus. We did not see any signs of differentiation of the cells at this stage. There are no traces of sensory hairs on the apical surface of the receptor layer.

The cells of the subjacent ganglion have large, round or slightly oval nuclei with large, dark nucleoli, rich in protein and carboxyl groups of protein molecules, and in nucleic acids. Their chromatin is very pale. The cytoplasm forms a very thin and uniform layer around the nucleus and is characterized by a very high RNA concentration, as well as considerable protein and carboxyl groups of protein molecules. The tufts of nerve fibers, also developing at this stage, are somewhat poorer in these substances than is the cytoplasm (Fig. 13).

With the appearance of teeth and the closing of the branchial fold, when the tadpole becomes somewhat more active (stages 23-24), the process of differentiation begins in the receptor epithelium of the maculae and cristae. The nuclei composing their cellular elements have increased considerably in size. The clarity of the nuclear membrane and the DNA concentration in the chromatin in the nucleus and nucleolus has decreased. The nucleoli no longer stand out against the pale background of the nucleus. As before, the receptor

epithelium contains many pigment granules, but they are mainly concentrated in the apical portion. Cytoplasm is visible only in the form of small particles in which the concentration of RNA, protein and its carboxyl groups is very low.

As before, the rudimentary ganglion consists of densely packed cells with round nuclei. It is mainly only the nuclei that are visible, since all of the cytoplasm (as before) is literally stuffed with granules of pigment. The nuclei of the ganglionic cells are much paler than the nuclei of the receptor epithelium, which has to do with the very low concentration of DNA in them.

The concentration of total protein is the same as that of nucleic acids. However, as in the case of the nuclei of the receptor epithelium and the nuclei of the ganglionic cells, it is not very clearly defined; the nucleoli still stand out with their much higher protein concentration, although the latter differs in individual cells.

Against this background, it is completely unexpected to find a very high concentration of carboxyl groups in these formations. These groups are found in the nucleus, not only in the chromatin and nucleolus, but in the karyoplasm as well, where the same concentration of carboxyl groups is found as in the cytoplasm. The chromatin appears only slightly darker than the karyoplasm. In the central portions of the macula, the nucleoli stand out very sharply, with an extremely high concentration of carboxyl groups in them. The nucleoli in the peripheral areas of the macula appear more pale. A still higher concentration of carboxyl groups is observed in the cells of the subjacent ganglion. Their large, slightly oval nuclei stand out against the completely colorless karyoplasm. The individual ganglionic cells differ slightly from one another with respect to the intensity of the reaction in the cytoplasm. In the majority of cells, /100 the nuclear chromatin has a very high concentration of carboxyl groups, with the maximum found in the nucleolus. In some cells, the nucleoli are located eccentrically in the nucleus. Rather considerable amounts of cytoplasm can be seen around the nucleus, but the cell boundaries are difficult to see. This is due mainly to the many pigment granules.

The same concentration and distribution of carboxyl groups is found in the sensory epithelium of the crista.

At the 25th and 26th stages (according to Terent'yev), i.e., at the time of the final formation of the branchial cavity and the appearance of the undifferentiated rudiments of the posterior extremities, division of the receptor cells is in progress in the sensory epithelium of the maculae and cristae. The cells in the epithelium are arranged less densely, and the cell boundaries are quite visible. The upper part of the epithelium contains cells with larger nuclei (future receptor cells), while the basal portion of the receptor layer contains the finer nuclei of the supporting cells. Pigment gran-

ules occupy the apical portions of the cells in the central areas of the maculae and cristae, but they are packed less densely than in the preceding stages. The processes of differentiation of the future receptor cells begin in the central portions of the terminal organs and gradually move toward their periphery.

The receptor and supporting cells do not differ with respect to the distribution and concentration of nucleic acids. The concentration of nucleic acids in the chromatin and in the nucleolus is average, and somewhat less in the cytoplasm. A particularly interesting and characteristic feature of this stage is the diffuse coloration of the karyoplasm of both the sensory and supporting cells; while staining with galloxyanin indicates the presence of RNA, staining with methyl green and the presence of positive coloration in the Fel'gen reaction would indicate the presence of diffuse DNA, with which finding it is difficult to agree. However, we observed this repeatedly. In performing the Fel'gen reaction, the stained karyoplasm is accompanied by completely colorless cytoplasm so that there can be no question of a non-specific reaction. In the sensory epithelium, the fine nuclei of the supporting cells show a higher concentration of DNA in the chromatin.

In these preparations, the karyoplasm in the nuclei of the cells in the subjacent ganglion of the eighth nerve are always colorless. Their chromatin has a very weak concentration of DNA. The nucleolus is characterized by a high concentration of DNA and a low concentration of RNA. A slight staining of the karyoplasm during the reaction with galloxyanin is observed in the small nuclei of the ganglion, which apparently belong to small cell-satellites. As far as the concentration of RNA in the cytoplasm of the ganglionic cells is concerned, it is very difficult to state anything definite, since it is completely filled with pigment granules.

The concentration of total protein rises sharply during these stages. The nuclear membrane, chromatin, and nucleolus are particularly rich in this substance. The concentration of the carboxyl groups also remains high, while in this reaction the sensitive hairs (containing a low concentration of carboxyl groups) first become visible in the macula at the tops of the receptor cells. A higher concentration of them is found in the chromatin of the nuclei of the receptor cells; it is still higher in the nucleolus, and highest in the nuclear membrane. No otoliths or otolith membrane are visible in fixed preparations. The ganglion increases in size relative to earlier stages, but the distribution in it remains the same, except that the concentration of carboxyl groups is somewhat less in the chromatin and in the nucleolus.

The concentration of thiol groups is somewhat higher relative to earlier stages. The difference between their distribution in the sensitive epithelium and the distribution of carboxyl groups lies in the fact that they have the same concentration in the nucleolus and in the chromatin. The distribution of thiol groups in the future

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ganglionic cells coincides with that of the carboxyl groups.

During the 27th and 28th stages, when the development of the posterior extremities occurs and the motility of the larva increases, basic processes of differentiation of the sensory epithelium are taking place in the inner ear.

The most significant changes occur in the neurons of the ganglion of the eighth nerve, taking the form of an increase in size of the cell body by expansion of the cytoplasm. As before, pigment granules are retained in the cytoplasm, but thanks to the considerable increase in its volume, the density of these granules is comparatively low. The nuclei in the neurons gradually assume an eccentric position. The concentration of RNA in the cytoplasm rises sharply. It is difficult to ascertain whether there is any tigroid in the cytoplasm since the pigment granules obscure it. The protein reaction reveals the nuclear structures to be extremely rich in protein. The eccentrically located nucleoli are very rich in proteins and their functional groups (Fig. 14). The body of the cell assumes a characteristic teardrop shape. The cytoplasm shows a high concentration of protein and its functional groups, but their distribution is uniform.

The receptor epithelium of the maculae and cristae undergoes a fine cytoplasmic differentiation, expressed in the development of the sensory hairs and the cuticular plates on the apical end of the receptor cells. One has the impression that the processes of differentiation in the sensory epithelium of the crista slightly anticipate those in the sensory epithelium of the macula. In these stages, then, the concentration of total protein in the macula is very high and more or less uniform, while in the crista the concentration of protein falls and it is distributed in the receptor layer in a more diverse manner. The maximum concentration is in the cuticle, the minimum in the hairs. At later stages, the protein concentration in all receptor structures of the inner ear decreases and their locations become more diverse, i.e., becomes more like that which we have seen at earlier stages in the crista. The differentiation processes gradually spread all through the receptor layer, occupying both the central and peripheral portions of the macula. In the terminal organs of the frog, however, there are no such sharp cytochemical differences between differentiated and undifferentiated areas such as we found in the differentiation of the maculae and cristae in the inner ear of the groundling.

Prior to the beginning of metamorphosis, the structure of the epithelium of the maculae and cristae shows practically no difference from the definitive form. In the central portions of the macula, the sensory epithelium is arranged in three layers (Fig. 15). The top third of the epithelium contains a layer of receptor cells with larger nuclei. The apical portions of these cells contain pigment granules. At the top is the cuticular plate and some rather long sensory hairs. The bases of the receptor cells do not reach the

basilar membrane, and are located above the middle portion of the epithelium. Their cell membrane, however, is practically indistinguishable from the cytoplasm, and the outline of the cell body cannot be seen clearly. About two-thirds of the sensory epithelium (in thickness) is occupied by the bodies of the supporting cells. Their smaller and more oval nuclei are arranged in 1 or 2 rows. The cell membrane is practically indistinguishable. The sensory epithelium of the crista has a generally similar appearance, and differs mainly in the longer sensory hairs.

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The ganglion of the eighth nerve has a definitive structure. Its neurons have moved far from each other, and each cell is surrounded by a capsule of cell-satellites. Numerous nerve fibers are visible. The cells have a typical bipolar shape, but the cut usually passes through one of the branches. In the main, they have round nuclei with one (not always clearly visible) nucleolus. In the majority of ganglionic cells, the nuclei occupy a central position. In some cells, some not very clearly outlined Nissl's substance can be seen, but it is considerably obscured by the pigment granules in the cytoplasm.

In our study of nucleic acids, the highest concentration of DNA was found in the small nuclei of the supporting cells of the maculae and cristae; their karyoplasm is also stained. In the larger nuclei of the receptor cells, the karyoplasm is clear, the chromatin shows the complex structure of the nucleus, and the DNA concentration in it is rather low. The nucleolus in the receptor cells is hard to see. The RNA concentration in the cytoplasm of both the receptor and supporting cells is very low. It reaches an average level in the apical portions of the cytoplasm of the receptor cells, but here the large number of pigment granules obscures it. The sensitive hairs are not stained specifically by pyronine. The distribution and concentration of nucleic acids is the same in the different reactions for nucleic acids. The total protein has a similar distribution and concentration.

A very clear picture is obtained in analyzing the sensory epithelium for carboxyl groups of protein molecules. In this reaction, the difference between the receptor and supporting cells of the maculae and cristae becomes very clear. While the concentration of carboxyl groups in their nuclei is approximately the same, it is much higher in the cytoplasm of the receptor cells than in that of the supporting cells. Hence, the bodies of the receptor cells form a line which stands out sharply due to the darker coloring of the cytoplasm. The bases of the receptor cells are arranged on more or less the same level. The long sensory hairs are distinguished by a low concentration of carboxyl groups. The concentration of carboxyl groups is higher at the apical ends of their cytoplasm. The cytoplasm of the supporting cells, which is poorer in carboxyl groups, makes their dark nuclei very noticeable. The cuticle and basilar membrane are distinguished by their higher concentration of carboxyl groups.

The distribution of thiol groups in the sensitive epithelium is very uniform. It is the only protein reaction which does not indicate increased concentration in the apical portions of the cytoplasm of the sensory cells. The concentration of thiol groups in the nucleus is less than in the cytoplasm. The sensory hairs are clear. The cuticle and basilar membrane do not differ from the cytoplasm as far as the concentration of thiol groups in them is concerned.

The subjacent ganglion of the eighth nerve shows a low concentration of DNA in the nuclear chromatin and a higher one in the nucleolus. On the other hand, the concentration of RNA in the nucleolus is very low; in some cells, the nucleolus appears to be empty after treatment by the Brach method. The cytoplasm of the neurons is rich in RNA, which is distributed more or less homogeneously through it. In some cells it is possible to assume the presence of tigroid, but the pigment granules make it very difficult to reach a final conclusion on this point.

A very interesting picture is provided by the distribution of the total protein. The cytoplasmic cell membrane shows up very clearly in the large nerve cells. In some cells, it is possible to see very great differences in the concentration of total protein. In some cells, tigroid is clearly visible. The nerve fibers running from the cells have a lower concentration of protein relative to the cytoplasm of the cell itself.

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The carboxyl groups are most richly represented in the cytoplasm and in the nucleolus. For the rest, the pattern of their distribution is more or less uniform.

In analyzing the preparation for thiol groups, the structure of the nuclei of the ganglionic cells shows up clearly. The concentration of thiol groups in the cytoplasm of the ganglionic cells is higher than in the cytoplasm of the receptor cells.

In the case of amphibians, the reaction for glycogen does not give any reliable results at all, because the numerous pigment granules obscure any changes. One has the impression that the glycogen literally fills the entire cytoplasm of the receptor cells at the earlier stages, but that its quantity decreases somewhat later on. A more or less visibly diffuse coloration of the cytoplasm of the receptor cells can be seen; as far as the granular inclusions are concerned, they cannot always be distinguished from the pigment granules (Fig. 16). Not long after the beginning of metamorphosis, a definitely positive Schick reaction occurs in the apical portions of the receptor cells. The basal accumulation of glycogen in these cells then decreases.

The process of development of the inner ear in amphibians is characterized by several chronological interdependencies among the differentiating receptor organs besides those that occurred in the development of the inner ear of the groundling. In the frog, the

cytological differentiation of the cristae occurs somewhat earlier than the cytological differentiation of the maculae, although both appear to be completely differentiated at the beginning of metamorphosis. This phenomenon of heterochronia is evidently linked somehow to the emergence of the frog onto dry land and the increase of its mobility in various directions. It is possible that this assumption is not completely warranted, since the data of several authors (Kolmer, 1927) indicate that such features are far from characteristic of all representatives of this class; on the basis of the very limited amount of material we had available for comparison, it is difficult to link the heterochronia with the ecological features of the existence of a given form.

Another distinctive feature of the development of the inner ear of the frog is the presence of yolk grains and a large amount of pigment grains in the cells of the auditory vesicle, which are retained for the entire duration of embryonal development. Their role and significance are unknown at the present time.

As far as we can judge from the actual material on hand, the histochemical regularities of the differentiation of the receptor structures of the inner ear in amphibians and their cellular structure generally do not differ from those of ganoid and teleost fishes. However, a low concentration of nucleic acids, protein and functional groups of protein molecules is characteristic of the differentiated cells of the inner ear of the frog prior to the beginning of differentiation. Their receptor cells do not have a sufficiently distinct cytochemical characteristic, which coincides with their morphological polymorphism in the receptor structures of the inner ear at definitive stages. Rather long sensory hairs also appear in the receptor cells of the macula, which is difficult to explain from the functional standpoint.

More definite and characteristic patterns are found in the differentiation process of the neurons of the ganglion of the eighth nerve; this can evidently be explained primarily by the significant increase in the size of the cell body as well as the marked spatial changes in localization and concentration of the chemical and biologically active substances.

DEVELOPMENT OF THE INTERNAL EAR OF REPTILES

The early stages of development of the marsh turtle are not readily available for investigation: when the egg is laid, it already contains an embryo measuring 6 to 7 mm from the parietal area to the coccyx, i.e., with a well-developed auditory vesicle. In the still undifferentiated epithelium of the thickened wall of the auditory vesicle, forming the macula communis, there is an average reaction to RNA and an intense one to DNA, total protein and functional groups of protein molecules. An exceptionally high reaction intensity is found when determining carboxyl groups. When the macula communis divides, the intensity of these reactions decreases considerably.

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In turtle embryos measuring 10 mm from the parietal area to the coccyx, two parts are clearly visible in the auditory vesicle, the pars superior and the pars inferior, as well as the rudiments of two auditory spots (maculae). In fixed preparations, however, the covering portion of the auditory vesicle appears to be severely twisted and it is difficult to say with certainty whether or not there is any rudiment of the semicircular canals at this stage. Although the larger part of the rudiment of the internal ear is in the form of a membranous wall consisting of cubic epithelium, a considerable part of it is occupied by the rudiment of the receptor epithelium. A capsule of condensed mesenchyma, in some parts of which the process of cartilage formation has already begun, is beginning to form around the rudiment of the inner ear. However, no spaces for the semicircular canals are yet visible in this capsule.

The rudiment of the receptor epithelium is clearly evident, but there is still no clear morphological difference in the structure of the cytoplasm and nuclei between the receptor and supporting cells at this stage.

The upper third of the rudiment of the receptor layer consists of the supernuclear cytoplasmic portions of the cells composing it. Their cytoplasm is marked by an insignificant concentration of RNA, but the latter is still higher than in the cytoplasm of the cells of cubic epithelium composing the remaining part of the wall of the auditory vesicle. The lower two-thirds of this rudiment (in thickness) consists of the parts of the cells which contain the nuclei. The cytoplasm here is arranged in a very thin layer and is nearly invisible beneath an optical microscope; the cell boundaries can be traced only with difficulty and only those nuclei that lie close together can be seen distinctly. They are large in size and irregular in shape. The stained karyoplasm contains 1 or 2 large, very intensely stained nucleoli. The basilar membrane was not examined in the reaction for nucleic acids.

In the reaction for total protein, the largest concentration of the latter was found along the apical edge of the rudiment of the receptor layer, in the region of the base of the otolith membrane. The latter has the form of individual small vesicles or lumps, which differ from the subjacent cells in having a very high concentration of total protein, and not in the form of a flat layer as in other vertebrates. A high concentration of total protein is seen also in the nuclear membranes and on the periphery of the nucleolus in the cells of the rudiment of the receptor epithelium. The chromatin in these nuclei takes the form of fine granular accumulations. The karyoplasm has a slightly yellowish hue. An average concentration of total protein is characteristic of the cytoplasm of the apical portions of the cells, with a slight intensification in the perinuclear area.

The rudiment of the vestibular ganglion is made up of relatively small cells with dark nuclei and lighter cytoplasm. On the whole,

the nucleus produces an intense, diffuse reaction to protein, and it is very difficult to distinguish the structure in it.

The very high concentration of carboxyl groups is found in the nuclei of the cells in the receptor epithelium, where they (like the total protein) are distributed diffusely in both the karyoplasm and the chromatin. The concentration of carboxyl groups in the cytoplasm of these cells is significantly lower than in the nucleus. /105

In the reaction to the thiol groups for this stage, it is characteristic that an insignificant concentration of sulhydryl groups is observed with a high concentration of total thiol groups in the cells of the rudiment. A second property of the distribution of the thiol groups is their uniform concentration in the cytoplasm and nucleus of the undifferentiated cells and the disturbance of this equilibrium in favor of the nuclei at the start of the differentiation process. In all cases, it is difficult to trace the structure of the nuclei and cell boundaries in the reaction to thiol groups in all cases in the rudiment of the receptor epithelium at this stage.

In turtle embryos 14 mm long, it is easy to see the rudiments of the semicircular canals, with the epithelial rudiments of the cristae, which have separated from the auditory vesicle, although the expanding ampules still do not show up clearly. The macula sacularis and macula utricularis already stand out from the rest of the wall of the inner ear at this stage and appear to be fairly well formed from an anatomical standpoint. In the epithelial rudiment, the division of the cellular elements into the future receptor and supporting elements is already under way, although this process, unlike that in other vertebrates, does not take place in the central portion of the macula but in several areas which are separate from one another, and which act as "centers of differentiation" for each macula. The nuclei of the receptor cells appear somewhat larger than the nuclei of the supporting cells, having a more or less regular rounded shape, a clear karyoplasm, and chromatin in the form of fine grains. The cytoplasm in the apical portions appears slightly fibrous. Here individual mitoses are visible, located close to the cavity of the inner ear. The supporting cells are packed very closely together. Their round or rounded-oval nuclei appear darker and smaller than the nuclei of the receptor cells. The chromatin in them is gathered in large lumps.

In these parts of the macula utricularis in which division into receptor and supporting elements has not yet taken place, the cells appear uniform and their nuclei have a narrow, elongated oval shape with dark, sometimes large nuclei and dark grains of chromatin, both large and small.

The rudiment of the gelatinous portion of the otolith membrane

is located above the tops of the cells in the rudiment of the sensory epithelium in the form of a narrow, homogeneous strip. No otoliths are yet visible at this stage (Fig. 17).

The receptor epithelium of the rudiment of the crista gives the impression of being less differentiated at this stage than it is in the rudiments of the macula utricularis and the macula saccularis, which actually do not differ from one another at this stage. While in the receptor epithelium of the macula utricularis the tops of the cells form a very flat area, which evidently has something to do with the development of desmosis and is one of the signs of incipient differentiation of the epithelial receptor layer, in the rudiment of the crista the positions of adjacent cells do not coincide. The cells of the rudiment of the crista have dark cytoplasm and very uniform dark nuclei of elongated oval shape, sometimes with narrowed or slightly bent ends. The basilar membrane cannot be seen. The morphological picture and histochemical characteristics of these cells coincide with those of undifferentiated parts of the epithelial rudiment of the macula utricularis, so that the description of the histochemical picture will apply mainly only to the rudiment of the receptor epithelium of the macula utricularis.

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Around the rudiment of the inner ear, one can see the auditory capsule, in a considerable part of which the processes of cartilage formation are already in progress.

In the reaction for nucleic acids, a high reaction intensity is observed in the less differentiated parts of the rudiment, thus indicating a high concentration of nucleic acids in them. Hence, the maximum concentration of nucleic acids is found in the rudiments of the receptor epithelium of the cristae and in the undifferentiated parts of the macula utricularis and macula saccularis. In all rudiments of the receptor epithelium, however, the intensity of the reaction to DNA far exceeds that to RNA and always remains at a very high level. Even in the nucleoli of the receptor cells, the concentration of RNA is significantly less than the concentration of DNA. The basilar membrane in the macula utricularis, during the reaction to nucleic acids, is visible in the more differentiated areas in the form of a colorless strip. The concentration of nucleic acids in the cubic epithelium of the remaining part of the wall of the membranous labyrinth is close to that in the rudiment of the receptor epithelium of the cristae (Figs. 17 and 18).

In the rudiment of the ganglion at this stage, the nuclei of the individual nerve cells appear to be much larger and have a vesicular shape. The karyoplasm of the nuclei is clear. Chromatin appears in the form of very fine granules and has a very weak concentration of DNA. The cytoplasmic portions of the ganglionic cells are relatively small, but very rich in RNA.

The content of total protein in the entire receptor epithelium at this stage is not very great, but in those cells which are located

at the "centers of differentiation" its concentration drops noticeably; this is especially striking in the nuclei of the receptor and especially the supporting cells. As far as the cytoplasm of the cells is concerned, the equality of the distribution of the total protein is disturbed; the concentration of the latter increases significantly at the apical ends of the cells. There is a considerable difference in the concentration of protein between the apical and basal portions of the cytoplasm in the supporting cells, while the sharp increase in the protein concentration in the apical portions of the cytoplasm is apparently connected with the onset of desmosis. Under the optical microscope, during the reaction for total protein, it is possible to see very intensely stained triangles (desmosomes) at the tips of such cells, which are considerably narrowed in the apical region. The nuclei of the receptor cells contain fine yet dark nucleoli which are difficult to distinguish from chromatin. In the upper rows of nuclei of the supporting cells, the nucleoli are large and sometimes irregularly shaped, while the DNA in them is richer in protein than the RNA. In the rows at the base, the nuclei of the supporting cells are clear. Their nucleoli are more pale and practically indistinguishable from chromatin. Sometimes the nucleoli are in the form of rings. The entire basal portion of the sensory epithelium, densely packed with nuclei, is much clearer than the apical portion, which is nearly free of nuclei. A cuticle has evidently formed on the tops of some cells. The rudiment of the otolith membrane, in the form of a narrow dark strip, lies above the tops of the cells of the sensory epithelium. It appears slightly wavy, with each "wave" corresponding to the width of the subjacent cell. In the basal portion of the sensory epithelium, one can see the afferent nerve fibers which then become lost among the cell boundaries, which have the same coloration.

In the undifferentiated parts of the macula utricularis, where the division of the cell elements into receptor and supporting structures has not yet taken place, the nuclei of all the cells (as we have already pointed out) have the same oval shape and the concentration of total protein in them is the same as in the cytoplasm of these cells. The structure of such nuclei is quite easy to follow, due to the much greater concentration of protein in them than in the karyoplasm. A still higher concentration of protein is found in the nucleolus. It should be pointed out that a portion of these cells, which do not differ morphologically or cytochemically from their neighbors, are located higher than the basic mass of cells in the receptor epithelium. This moves us to suggest that during the process of separation of the common cellular rudiment into the receptor and supporting elements, spatial shifting of the cells precedes the morphological and cytochemical changes, which may be related to the freeing of the bases of the cells and the contraction ("tightening") of their cytoplasm and the apical portion of the epithelium, which leads to a shift of the entire cell body to the apical part of the epithelial layer. However, in view of the fact that the cell boundaries, especially in the basal portion of the epithelium, cannot be followed in the early stages of development, it is

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not possible to study this process in detail. It is only possible to suggest what occurs, on the basis of some indirect information and the final results.

The otolith membrane is visible above the apical surface of the cells in the form of a structureless mass, marked by an average concentration of total protein. Above the single-layered cylindrical epithelium bordering the macula, one can see the vesicles of secretion which give a weak protein reaction. They are very similar to the substance of the otolith membrane as far as the nature of the protein reaction is concerned.

The reaction to the sulhydryl groups is quite intense at this stage. The cytoplasm appears fibrous. The apical portion of the cytoplasm is free of nuclei and much darker than the remainder of the epithelium, occupied by nuclei. The structure of the clear nuclei is highly visible. The nucleoli in them are much darker than the cytoplasm. In the central portions of the macula, one can see short and long pitcher-shaped cells, which were not revealed by other reactions. In the short pitcher-shaped cells one can easily see cup-shaped synapses with very clear neuroplasm and clearly marked protoplasmic membrane. Also visible are the afferent nerve fibers in the receptor epithelium and their paths to the receptor cells. However, only a few pitcher-shaped cells with cup-shaped synapses can be seen.

The neuromas of the rudiment of the vestibular ganglion, in the reaction for thiol groups, are marked by the clear karyoplasm of the vesicular nuclei, very dark nucleolus, and clear chromatin. The cytoplasm of the cell body is much poorer in thiol groups than the nerve branches running from it. An especially high concentration of thiol groups occurs in the nerve fibers running to the receptor epithelium, so that they appear dark and very distinct. In the nerve bundle, between the dark nerve fibers, one can see the narrow, bright nuclei of Schwann's glia.

The carboxyl groups in this as in all subsequent stages of development (turtle embryos measuring 14 to 23 mm from parietal area to coccyx) are found in very high concentration both in all rudiments of the receptor formations of the inner ear and in the rudiment of the vestibular ganglion. Their concentration is so great that it completely obscures the fine structure of the cells. A slight drop in their concentration can be observed only beginning with the 23 mm stage.

In subsequent stages, the processes of differentiation of the receptor structures of the inner ear lead mainly (1) to a spread of the differentiation processes to the still undifferentiated parts of the receptor epithelium and (2) to further processes of the thin structure of the specific differentiation of the receptor and supporting elements. The latter is expressed in the formation of sensory hairs and cuticular plates, the development of cytoplasmic

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organoids and the synaptic apparatus in the receptor cells, as well as the reticular membrane, tonofibrils, etc. in the supporting elements. These two directions of the processes of differentiation are also observed in the rudiment of the vestibular ganglion. All of these morphological changes are accompanied by a change in the concentration and localization of the biologically active substances.

In turtle embryos 18 mm long, in those parts of the macula utricularis which we referred to above as "centers of differentiation", one can see the beginning of formation of individual Type I receptor cells, in which the cup-shaped synapses develop. However, the number of such cells is still very small. Insofar as the sensory epithelium of the cristae is concerned, it has an undifferentiated appearance as before.

The intensity of the reaction to RNA in the sensory epithelium of the differentiated portions of the macula decreases somewhat. There is no RNA in the cup-shaped nerve endings. Well-developed sensory hairs are clearly visible at the tops of the receptor cells. In the internal lumen of the labyrinth, there is a large number of characteristically shaped otoliths, large, small, and medium in size. The intensity of the reaction to DNA remains at the former level, and the structure of the nuclei of the receptor and supporting cells remains unchanged (Fig. 19).

The nerve cells in the vestibular ganglion increase considerably in size, due mainly to the increase in cytoplasm volume. The latter is slightly vacuolized and gives a very intense reaction to RNA. The vesicular nucleus contains rather pale, fine-grained chromatin in the periphery. In the center of the nucleus, there is a nucleolus with an unusually high RNA content. In the cells of the capsule, the cytoplasm is very pale, nearly invisible, while the small nuclei are unusually rich in DNA (Fig. 20).

The cartilaginous capsule around the membranous labyrinth is completely developed at this stage. In its cavity, in the immediate vicinity of the receptor structures, there is a large number of large blood vessels.

In studying the total protein in the receptor cells of the macula, we see a rather low concentration, which does, however, increase to average levels in the apical and subnuclear portions of the cytoplasm. The nuclei of the receptor cells remain very clear, with a high reaction intensity in their nucleoli and in the nuclear membrane. Against the background of the general mass of cells in the receptor epithelium, one can see very clearly the darker, pitcher-shaped receptor cells with very pale cup-shaped nerve endings. The gelatinous substance of the otolith membrane and the supporting cells show a very mild reaction to protein. The otoliths remain completely colorless.

In the vestibular ganglion at this same stage, only the nuclear

membrane and nucleoli yield a very intense reaction to total protein. The chromatin of the nucleus and cytoplasm shows a uniform mild reaction. In contrast to the situation with nucleic acids, an extremely mild reaction occurs in the nuclei of the cells in the capsule.

As mentioned earlier, the concentration of carboxyl groups of protein molecules is extremely high in the neurons.

The reaction for the total occurrence of thiol groups in the receptor structures of the internal ear proceeds very intensely. The cytoplasm of the supporting cells of the macula utricularis contains an average concentration of thiol groups. Their concentration is noticeably greater in the cytoplasm of the receptor cells. The nuclei of the receptor cells are clearer, with dark nucleoli. The large cup-shaped nerve endings exhibit a weak reaction to thiol groups. A very high concentration is found in the basilar membrane (Fig. 21a). /109

When testing only for a few of the sulfhydryl groups, the sensory epithelium of the macula appears much more pale. The nuclei of the supporting cells appear very clear. The structure of the receptor epithelium is poorly visible in this reaction, however. In the pitcher-shaped receptor cells, the nuclei are cloudy and dark, as is the cytoplasm of these cells. The apical portion of the cytoplasm appears darker than the basal (Fig. 21b).

The structure of the vestibular ganglion is very clearly visible in the reaction for thiol groups. The capsule and nerve fibers stand out sharply. The most intense reaction is observed in the nuclear membranes and in the nucleoli. The cell membranes, on the other hand, can be seen through only with difficulty. A very high concentration of thiol groups is found in the cytoplasm of the neurons. The efferent nerve branches appear to be clearer. The nuclei of the capsular cells remain very clear. The reaction to sulfhydryl groups is of much less intensity.

In all the stages described above, and in parallel with the process of differentiation of the receptor epithelium of the maculae and cristae, there is a very great increase in the volume of the inner ear, due mainly to the growth of its membranous wall. This increase in all probability occurs not only through mitotic division of the cells in the epithelial lining, but by stretching of the wall of the inner ear. In any case, the ratio between the receptor and lining epithelium clearly changes in favor of the latter. In the final stages of development, the structure of the membranous labyrinth of the turtle, in this respect, approaches that of the membranous labyrinth of teleost fishes.

At the 23 mm stage, the embryo of the marsh turtle has an inner ear whose structure is nearly definitive. The process of differentiation at this stage is essentially complete in both the maculae and cristae.

The RNA content in the cytoplasm of the receptor and supporting cells of the sensory epithelium, which are approaching completion of their cytological differentiation, is very low. It increases somewhat in the apical portions of the receptor cells. In the reaction for nucleic acids, the cell boundaries are difficult to see through. The nuclei of the supporting cells retain their former shape and their previous (quite weak) intensity of reaction to DNA. Their nucleolus is rich in RNA. The rather fine nuclei of the supporting cells are arranged in one or two rows in the basal portion of the receptor epithelium. They are very densely packed and are distinguished by a high reaction intensity toward DNA. RNA is not visible in their nucleoli (Fig. 22).

In the crista, the sensory epithelium is found only at the top of the crest. The side walls of the crista are covered with the usual cubic epithelial lining. In the receptor epithelium of the crista, the intensity of the reaction to RNA is somewhat less than in the macula.

In the ganglionic cells of the vestibular ganglion, there is a reaction of low intensity for both DNA and RNA. In the cells of the capsule, the concentration of nucleic acids is much higher (Fig. 23).

The protein reaction in the sensory epithelium is very intense, with the greatest intensity found in the cytoplasm and nucleus of the receptor cells. The structure of the nucleus is nearly indiscernible, since the high concentration of protein occurs in the karyoplasm as well. The supporting cells differ only insignificantly from the receptor cells with respect to the protein content. The lowest protein content is found in the cup-shaped nerve endings.

In the ganglion, an exceptionally rich supply of protein is found in the nuclear membrane, in the periphery of the nucleolus, and in the cytoplasm of the neurons. A lower concentration is found in the cytoplasm and in the nuclei of the capsular cells. A low concentration of protein is also found in the nerve fibers. The carboxyl groups are marked by a very high concentration in all structures of the internal ear and in the vestibular ganglion, although at this stage it is still possible to see a very small drop in their concentration. /110

In terms of the overall picture for thiol groups, a high concentration of them is found in the maculae and cristae. The cytoplasm of the receptor cells is extremely rich in thiol groups. Their nuclei have a moderate concentration, which is the same in both the karyoplasm and the chromatin. A mild reaction is seen in the cup-shaped nerve endings and in the nuclei of the supporting cells.

The concentration of sulfhydryl groups differs insignificantly in intensity from the total reaction for thiol groups, and their locations coincide.

In summing up, we can say that the most characteristic moment in the differentiation of the receptor formations of the inner ear of the marsh turtle may be considered to be the appearance of the pitcher-shaped receptor cells (so-called Type I cells) with cup-shaped synapses, along with cylindrical receptor cells (Type II cells) with bud-shaped nerve endings. The appearance of these cells (Wersäll, 1956) indicates that the vestibular apparatus of reptiles can detect not only general, scattered effects but also finer, local effects of the gravitational field. In the course of the embryogenesis of reptiles, the pitcher-shaped cells appear somewhat later than the cylindrical receptor cells and their occurrence is always linked with a change in the content and location of the biologically active chemical substances which we have studied.

A second outstanding feature of the inner ear of the turtle is the considerable increase in its volume, owing to the expansion of the utricle and saccule, while the semicircular canals remain small. At the same time, there is an increase in the size of the membranous parts of the utricle and saccule. However, this feature imposes its influence on the process of embryonal development, beginning with rather early embryonal stages of the auditory vesicle. In direct relation to this are the unusually large dimensions of the auditory vesicle even at the stage of division of the rudiments of the individual receptor organs and the very rapid and considerable increase in the size of the walls of the saccule and utricle at later stages of development, as well as the later formation of the semicircular canals relative to the development of the inner ear in representatives of other classes of vertebrates.

There is still one more interesting feature of the embryogenesis of the inner ear of the turtle: the considerable chronological and developmental rupture of continuity at the beginning of differentiation of the maculae and cristae, as well as the existence of several centers of differentiation in the receptor epithelium of each macula.

From the viewpoint of cytochemical regularities, a characteristic feature of the developing internal ear of the turtle is the presence of an extremely high concentration of thiol groups in both the receptor epithelium and in the ganglion of the eighth nerve. The considerable changes in their concentration and location during embryonal development indicates that in contrast to fishes and amphibians, the thiol groups in the inner ear of the turtle play an active role in the differentiation of its receptor structures.

A characteristic feature of the final stages of differentiation /111 of the receptor epithelium is the low concentration of nucleic acids in the receptor cells, with a very high content of protein and functional groups of protein molecules in them. In this regard, there are considerable differences between the receptor elements and the neurons of the eighth ganglion, of which both a high concentration of protein and its functional groups and of nucleic acids are characteristic.

Development of the Inner Ear of Birds[†]

DEVELOPMENT OF THE VESTIBULAR APPARATUS

On the 4-th day of incubation, from which in fact our investigation began, the auditory placode is converted into the auditory fossa, which then, gradually deepening, is converted into the auditory vesicle, which originally preserves connection with the external medium by means of an opening. Toward the end of the 4-th day, closure of the auditory vesicle takes place as does its separation from the ectoderm (Fig. 24). At these stages in the wall of the auditory vesicle there is still no sign of cellular differentiation. Its cells have an elongated shape and pass through the entire thickness of the epithelial layer. The bases of the cell are located on the basal membrane, and the summits reach the apical surface of the vesicle. On preparations fixed with a Champi mixture, and then on preparations stained with ferric hematoxylin, it is possible to note darker and lighter cells containing, respectively, darker or lighter small oval nuclei. In the dark cells it is possible to trace the length of the cellular body from the apical surface of the epithelium to the basal membrane. The lighter cells have a less pronounced cellular body. They are very similar to cells of the rudiment of the ganglion adjacent to the wall of the auditory vesicle. The basal membrane of the epithelial layer, at the places adjacent to the rudiment of the ganglion, is not always clearly pronounced and in certain places patterns are seen which lead one to assume that a migration of cellular elements from the wall of the auditory vesicle into the underlying ganglion has taken place.

Nearer the internal surface of the auditory vesicle wall in dark elongated cells, a large number of thread-like mitochondria oriented parallel to the long axis of the cell is seen. In these nuclei, 1-2 nucleoli are visible. In the light cells, mitochondria are located in a less orderly fashion.

Cells of the ganglion rudiment are very pale with thin, delicate processes. Their round, irregularly shaped nuclei are poor in chromatin. Upon impregnation with silver, the cell processes are not silvered.

With electron microscope study the Macula communis of a 4-th day chick embryo is seen as a multinucleated layer of indifferent cells, rather loosely distributed (Fig. 25).

The surface of the epithelium is uneven due to the fact that the summits of the cells have an irregular shape. A cell is surrounded by a thin, one-layered protoplasmic membrane. Desmosomes are formed at the points of contact of the apical portions of

[†] Electron microscope investigation made together with F.G. Gribakim on chick embryos.

neighboring cells. In one of the portions near the surface, there is a suggestion of the presence of a backing of the kinocilium. However a study of this portion with great magnification by an electron microscope (30,000) did not completely clarify this question. Near this backing there are seen smooth membranes of the endoplasmic reticulum. At the summit of individual cells there is a backing of microvilli, which are formed by means of a protrusion of the single layered protoplasmic cellular membrane. /112

The cytoplasm of a cell is filled with numerous ribosomes, which in the majority of cases are freely distributed in a light cytoplasmic matrix. However, they settle on the membrane of the endoplasmic reticulum forming elements of a rough membrane. Most often these latter have the shape of single round vesicles or cisterns; more rarely they form long narrow profiles terminating in an expanded cistern. The latter elements are most often found in the basal portion of the cell. The endoplasmic reticulum of the cytoplasm of a cell basically consists of rough membranes with clearly visible but not very densely distributed ribosomes. In places, portions of the complex of Golgi's apparatus are seen located basically in the apical portion of the cell (Fig. 25).

In the cytoplasm of the cells of the *Macula communis* the presence of two types of mitochondria is observed: one type is rod-shaped or elongated oval mitochondria with dark matrix of high electron density with irregularly distributed densely packed cristae. The distribution of mitochondria in the cytoplasm of the cells is irregular. The greatest accumulations of mitochondria are observed in the basal portions of the cells, located on the basal membrane. The mitochondria here are primarily of the first type, i.e., dense and rod-shaped. Often there are mitochondria of irregular shape, sometimes extremely bent (Fig. 26). The mitochondria of the second type are often found in the apical portions of the cells (Fig. 25).

The basal membrane is not equally well defined along the entire length of the *Macula communis*. In the majority of cases only an internal osmiophilic single-layered membrane is seen, which appears as an external plasmatic membrane of the cells; a second external membrane is rarely noted and is formed due to the basic substance of connective tissue. Under the basal membrane there are cells of the ganglion rudiment and individual mesenchymic elements.

The nucleus of the cell is round-oval. The nuclear membrane is dual-contoured with a small open space. Its pores are very indistinct. The nucleus is filled with small granules of various electron density. The peripheral chromatin is always found in close contact with the internal nuclear membrane. The 1-2 nucleoli are of irregular shape and consist of very small granules of high electron density. In individual cells there are found "perforated" nuclei, which possibly are connected with the migration of ribosomes to the periphery of the nucleus.

Upon testing for nucleic acids, the wall of the auditory vesicle at these stages appears under a light microscope to consist of high cylindrical multinucleated epithelium. Upon staining according to Brachet's method, the nuclei of these cells seems to be dark due to intensive staining of the karyoplasm in a bluish-green color. Against this dark background, the structure of the chromatin is almost invisible. The nuclear membrane is pale; its contours are only traced with difficulty. The nucleoli are distinguished by an intense pyroninophilia which indicates that they contain a high concentration of RNA, and they are sharply distinguished against the dark green background of the nucleus. The average degree of specific staining of the cytoplasm by pyronin is increased in the region of the basal membrane and in the apical portion of the cell.

In the cells of the ganglion rudiment of the eighth nerve the nuclei are smaller and their karyoplasm is lighter. Dark grains of chromatin are easily seen as are the bright pyroninophilic nuclei. In a majority of cells of the rudiment the cytoplasm is bright and grainy. Staining of RNA with pyronin is specific and is eliminated by treating the preparation with ribonuclease. The described patterns are confirmed by investigations of DNA with the aid of Felgen's reaction and summary development of RNA and DNA with gallo-cyanin.

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The concentration of total proteins and functional groups of protein molecules is quite high. In addition, the greatest concentration of these is observed in the nucleolus.

In the auditory vesicle and in the rudiment of the ganglion of a 4-th day chick embryo glycogen is not detected histochemically. There are only certain mucopolysaccharides in the basal membrane and the backing of the covering formations of the inner ear receptor organs.

On the 5-th day of incubation the auditory vesicle is extended in the dorsal direction. The thickness of its walls changes: its medial portion begins to be thickened, and evidently this is the beginning of the formation of the Macula communis; the lateral wall, on the other hand, becomes significantly thinner. On preparations stained with osmium and hematoxylin, two types of cells stand out more clearly in the wall of the auditory vesicle, but this difference does not become apparent on histochemical preparations. This stage is interesting by the fact that in the ganglion there appear nerve fibers which may be impregnated with silver. The trace of the glial cells grows to the brain, and in it there are individual nerve fibers which may be impregnated with silver and fibers which may be stained with osmium proceeding to the brain and, possibly, entering it. However the wall of the auditory vesicle itself at the level of the medulla oblongata is still not differentiated at this time.

During the 5th-6th day of incubation the shape of the auditory vesicle changes. It is elongated in the dorsal direction and the

medial wall continues to thicken, i.e., the process of formation of the Macula communis begins. The remaining part of the wall of the auditory vesicle becomes significantly thinner and the epithelium composing it becomes a one-layered cylindrical, and then even cubical, epithelium. In the wall of the auditory vesicle the dark cells become very thin and the number of light cells significantly increases. A division of the auditory vesicle into two sections, the Pars superior and the Pars inferior, is noted. But these sections still communicate with one another by a broad opening. At the same time, with the separation of the auditory vesicle into two sections, there begins a division of the Macula communis into two corresponding portions. At this stage the neurofibers have still not entered the wall of the auditory vesicle. The processes of ganglion formation begin, and it acquires its characteristic outlines. The nuclei of the cells of the ganglion are well structured, but the cells themselves still have an unembryonal nature.

With reaction to nucleic acids, the large number of dividing cells located in the apical portion of the future receptor epithelium is especially apparent.

During the 4th-6th day of incubation it is possible to note the subsequent change in nucleic acids: on the 4-th day the auditory vesicle reacts to both RNA and DNA with equal intensity; i.e., the concentrations of DNA in the chromatin of the nucleus and RNA in the cytoplasm are on approximately the same level. On the 6-th day of incubation in the cells of the Macula communis a more intensive reaction to DNA than to RNA is observed, which indicates an elevation in DNA concentration in the chromatin and a decrease of RNA in the cytoplasm. If we compare the intensity of the reaction to DNA and RNA on the 4-th and 6-th days of incubation, then it becomes evident that the directions of the changes in nucleic acid concentration in the chromatin of the nucleus and in the cytoplasm do not correspond. While the concentration of DNA is elevated toward the 6-th day, the concentration of RNA in the cytoplasm falls. In the underlying rudiments of the ganglion we observed a somewhat different process: during this entire period the rudiment of the ganglion shows a significantly more intensive reaction to RNA in the cytoplasm than to DNA in the chromatin of the nucleus. Common to all these stages, both in the rudiment of the sensory epithelium and in the rudiment of the ganglion, is the invariably high concentration of RNA in the nucleolus. /114

On the 7-th day of incubation the configuration of the auditory vesicle is severely complicated. The rudiment of the inner ear increases in dimensions, and within its two sections the process of division of individual anatomical parts of the utriculus continues. So in the Pars superior the semicircular canals are established, and from the Pars inferior separates a short digitate outgrowth of the cochlear canal with the blind end of the lagena located on it. Both in the sacculus and in the utriculus one thickened wall appears as the rudiment of the receptor epithelium of the Macula. Inside the

sacculus and utricle, otoliths develop in a quite large number. Under a light microscope the receptor epithelium of the utricle differs little from the epithelium of the Macula communis. The future receptor cells are distinguished from the supporting cells only by their location.

With an investigation of the rudiment of the sensory epithelium of the utricle macula under an electron microscope, the abundance of cytoplasm of the receptor cell in ribosomes and large number of primarily rod-shaped, very dense mitochondria located in the cytoplasm of supporting cells nearer the basal membrane is conspicuous, although at this stage it is not observed over the entire length of the rudiment of the receptor epithelium of the utricle macula. The cytoplasm of receptor cells is also rich in mitochondria, but they are primarily round. In its cytoplasm an insignificant number of membranes of the endoplasmic reticulum is noted. The nucleus has a well defined double nuclear membrane, a fairly light matrix and a dark, finely granulated mass, uniformly filling the entire space of the nucleus. The nucleolus (sometimes there are several) has a significantly high electron density and is distinguished by the denser distribution of granular material composing it. The nucleolus usually consists of two parts of equal electron density; their mutual distribution may be different, but they always seem to be lying upon one another. Sometimes the nucleolus may occupy a marginal position lying close to the nuclear membrane. The shape and size of the nucleolus greatly varies. Sometimes there are segmented nucleoli (Fig. 27). The double nuclear membrane has the usual structure, but in several cells it is thickened due to the granules of nuclear substance densely attached to its internal side, and from the external side with cytoplasmic granules and, in all probability, ribosomes. Throughout the entire receptor layer the nuclei are homogeneous both in shape and ultrafine structure.

With Feigen's reaction the karyoplasm of the nuclei of the receptor layer is pinkish with a completely colorless cytoplasm. The concentration of DNA in the chromatin is average. It is intensely elevated in the chromatins of the dividing cells.

Upon staining with galloxyanin the intensity of the stain of the cytoplasm and the karyoplasm is equal. Against this background, the chromatin, nucleolus and contour of the nucleus stand out sharply. With a control treatment of the preparation by ribonuclease the stain of the cytoplasm is completely removed while the stain of the karyoplasm is preserved.

In neurons the intensity of the reaction to DNA at this stage is preserved, but the DNA loses its uniform distribution, being strengthened at the points of origin of the processes.

General or total protein is distributed very homogeneously in the receptor epithelium and has a very weak intensity of reaction. It is interesting that the intensity of reaction to total protein is

significantly higher in the lining epithelium in comparison with the receptor layer. Under the rudiment of the receptor epithelium is seen a stria rich in protein; i.e., the backing of the covering formations. In neurons the intensity of the reaction to protein is somewhat higher.

With the reaction to carboxyl groups the contours of the nuclei and the cellular ganglia stand out sharply. The intensity of reaction and the distribution of carboxyl groups in general do not differ from those with a reaction to total protein.

On the 8-th day of incubation nerve fibers begin to be well-impregnated with silver and to be stained with osmium. Peripheral processes of neurons are clearly seen, growing up to the sensory epithelium and entering it. But differentiation of neurons in the ganglion does not take place at the same time. In individual portions of the ganglion there are still neuroblasts. The most highly differentiated neurons have large vesiculiform nuclei with a large nucleolus and very small clumps of chromatin. The cytoplasmic body of such cells is comparatively small.

At this stage ampullae with anatomically well defined cristae are seen. Nerve fibers also grow up to the receptor epithelium, covering them. Due to the simple structure of such cristae and the absence of Septum cruciatum on them it is difficult to determine exactly to which semicircular canal a given crista belongs.

Thus at this stage all the anatomical parts of the inner ear have already developed, and the processes of fine cytological differentiation begin in it.

At the 8-th day of incubation a strengthening of the process of cellular differentiation in the receptor epithelium is cytochemically manifested by the sharp drop in DNA concentration and especially in RNA concentration. The level of nucleic acids in the ganglionic cells of the vestibular ganglion remains as before. On silvered and osmiated preparations it is possible to observe an ingrowth of nerve fibers into the receptor epithelium.

Changes in the receptor epithelium appear especially sharply with protein reactions. The apical portions of supporting cells stand out clearly due to the high concentration of protein in them and the presence of well defined longitudinal fibrillarity. Between them are very pale cells with round nuclei, evidently the future receptor cells. Above the sensory epithelium a layer of gelatinous substance which is poor in protein is seen.

The reaction for carboxyl groups as before indicates their high concentration, but they are still distributed equally in the epithelial layer. Above the surface of the sensory epithelium bundles of sensory hairs are seen in places. On the 9-th day of incubation glycogen appears in the cells of the vestibular ganglion, and toward

the 12-th day of incubation there is already a rather significant amount of glycogen in the cytoplasm of these cells. At these stages mucopolysaccharides are discovered in receptor epithelium of the maculae and in the cellular membranes, which indirectly attests to the increase in their permeability.

The process of differentiation of sensory and supporting cells continues even on the 10-th day of incubation. Besides the appearance of tonal fibrils in the supporting cells and a difference in the concentration of RNA and protein in the cytoplasm of receptor and supporting cells, differences arise even in the structure of their nuclei. The nuclei of receptor cells are increased in size and their chromatin acquires a marginal position. In several receptor cells nucleoli greatly increase in size. The nucleolus is most often located in the center of the nucleus but sometimes it is shifted toward the periphery of the nucleus and even completely attaches to the nuclear membrane.

Upon testing for nucleic acids, the karyoplasm of the nuclei or the receptor epithelium, as before, gives a diffuse positive reaction for DNA. In neurons of the vestibular ganglion, the concentration of DNA significantly increases, and a positive reaction for DNA in the karyoplasm appears.

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For this stage differentiated localization of carboxyl groups is characteristic. A clear reaction is given by the bands of gelatinous substance in the sensory epithelium, the apical portions of the cytoplasm receptor and supporting cells and the fibrils of supporting cells. The karyoplasm of the nuclei of receptor cells is more transparent and their cellular membrane and nucleoli are distinguished by a higher concentration of carboxyl groups than that obtained in the nuclei of supporting cells.

In the vestibular ganglion this reaction has differing intensities in its different portions. In those portions of the ganglion in which the reaction takes place more intensively there are individual, centrally located cells in which especially intensive reactions to carboxyl groups in their cytoplasm and nucleolus are observed.

On the 11-th day of incubation, on preparations of the utricular macula impregnated with silver, nerve fibers are seen ascending to the base of the receptor cells, on the summit of which there are sensory hairs. Intrusion of nerve fibers inside the sensory epithelium is observed even in the crista, but whether they reach the base of the receptor epithelium there could not be reliably established. In the central portions of the macula the receptor cells are more sharply differentiated from the supporting cells by the shape of the nucleus and by the cylindrically round shape of its cellular body. But all the receptor cells have the same shape.

Investigation under an electron microscope allows us to establish

that for the receptor epithelium of the utricle of an 11-day chick embryo the presence of completely formed bundles of sensory hairs containing 40-50 immobile stereocilia and 1 (in rare cases 2) mobile kinocilium is most characteristic. The stereocilia are primarily found in the shape of the letter W, in the notch of which is found the kinocilium. Sometimes even complex bundles of a different structure are detected (Fig. 29), but this process evidently is still not completely concluded since sometimes on individual cells it is possible to see only a kinocilium or a basal corpuscle without accompanying stereocilia (Fig. 29).

In the cytoplasm of the cell, in particular in its apical portion in the region of the future cuticle, there appears a significant number of elements of smooth endoplasmic reticulum. However the number of elements of the endoplasmic reticulum intensively increases with the simultaneous decrease in the amount of free ribosomes freely suspended in the cytoplasmatic matrix. The density of distribution of elements of the rough endoplasmic reticulum increases, whereupon the cisterns acquire a different shape. At the same time elements of the smooth reticulum, primarily in the form of narrow tubules, appear. In several cells it is possible to see elements of Golgi's apparatus (Fig. 30). Mitochondria located in the endoplasm of the cell are basically of a round shape, light matrix and a small number of loosely distributed cristae. Nerve fibers entering the epithelium are distinguished by the low electron density of the axoplasm, the absence of elements of the reticulum and the presence of an insignificant number of vesicles. For nerve fibers the presence of mitochondria with more densely distributed cristae is characteristic (Fig. 31).

On the 11-th day of incubation in the receptor epithelium of the macula and cristae a certain drop in the concentration of nucleic acids, chiefly RNA, is observed. This process is especially noticeable in the nerve cells of the vestibular ganglion. In several primarily central portions, the cells are increased in dimension, chiefly due to the vesiculiform nucleus. The latter is distinguished from the nuclei of receptor cells by its completely colorless karyoplasm and very pale, finely divided chromatin. The high concentration of both RNA and DNA is preserved only in the nucleolus; with the presence of two nucleoli in the cell they can have different concentrations of nucleic acids. The nucleus is usually located eccentrically in the cell. For the cytoplasm of the neurons of the vestibular ganglion an average concentration of RNA is characteristic, which in individual cells can be significantly lowered. /117

With a reaction for total protein and carboxyl groups of protein molecules, the receptor epithelium of the cristae is distinguished by the pale tone of the cytoplasmic stain having a fibrous nature. The apical portions of the cytoplasm are somewhat darker. All the nuclei have a very clear structure, nuclear membranes and chromatin barely darker than the cytoplasm. In cells of the vestibular ganglion the general tone of the stain is the same but the con-

tours of the nuclei in the chromatin are darker than in the sensory epithelium. With the reaction for carboxyl groups in the ganglion there are seen very dark nerve fibers. In the sensory epithelium of the crista a rather intense reaction to carboxyl groups is manifested in the sensory hairs. A very high concentration of carboxyl groups is observed in the apical portions of the supporting cells, in which is seen a longitudinal fibrillarity. Beside the crista there is a backing of epithelium of the Planum semilunatum, in which a significantly lower concentration of carboxyl groups is noted.

On the 12-th day of incubation, well-developed sensory hairs with otoliths lying above them are seen in the receptor epithelium of the macula. After fixation in Carnua's fluid, they are seen in the form of pale shadows. The difference between the utricular and saccular maculae consists not in the degree of differentiation of the cellular elements composing them, but in the extension of the portion where differentiated cells are located, which is smaller in the sacculus. From this it is possible to conclude that the processes of cellular differentiation in the saccular macula begins somewhat later.

On the receptor cells of the ampullar crista are seen very long and very thin fibers. As before, mitoses are seen in the apical portion of the receptor epithelium of the crista. With the reaction for carboxyl groups on the summit of the crista sometimes there are seen pitcher-shaped cells and even one group consisting of two pitcher-shaped cells located in one scyphoid synapse. The impression is created that the sensory hairs of the receptor cells are located on the cytoplasmic conuses. The thickness of the epithelial layer of the utricular maculae is somewhat increased. Receptor cells are densely located in one layer in the apical portion of the receptor epithelium. Their cytoplasm is denser and has a fibrous nature. Sensory hairs are shorter than on the crista but are also located on the cytoplasmic conus (1 per cell). The nuclei of supporting cells are small and oval in shape. In the central portions of the macula they are located in two rows. The apical portions of supporting cells have a threadlike shape and pass between the densely distributed bodies of receptor cells, reaching the surface of the receptor epithelium. The lower ends of the body of a supporting cell are broader, and rest on the basal membrane.

In the vestibular ganglion at this stage significant changes /118 take place. Large bipolar neurons are seen in it, and both processes very often do not fall into one section. These cells have large round nuclei with a very pale, scarcely noticeable chromatin. Cytoplasm in them is dark and strongly vacuolized. The nucleoli (one or two) are very pale.

Reaction for nucleic acids in the sensory epithelium does not give any new results. The features of all cellular elements stand out very clearly with protein reactions, which evidently is connected with the enrichment of their cellular membranes by proteins.

With these same reactions otoliths are seen but they are more similar to the membranes of the crystals than to the crystals themselves. With the appearance of carboxyl groups in the cells splitting by mitosis threads of the spindle are easily seen.

In nuclei of the neurons of the vestibular ganglion there is an exceptionally low concentration of DNA, protein and functional groups of protein molecules, with the exception of the nuclei in which the concentration of these substances is very high. Concentration in the distribution of RNA in the cytoplasm does not change (Fig. 32). Nucleoli are weakly pyroninophilic. In several cells there is preserved a weaker concentration of nucleic acids in the nucleolus. The cytoplasm of neurons is rich in RNA, protein and functional groups of protein molecules. On the 12-th day of incubation otoliths are stained very brightly in a cherry-red color; however, we did not succeed in establishing the nature of this staining with a SHIK reaction. At the same time inclusions of glycogen appear even in cells of the utricular macula and in very small quantities in the cells of the saccular macula. Granules of glycogen at the same time are located in the apical portions of the cells.

On the 13-th day of incubation a large amount of glycogen appears in the receptor cells of the utricular macula. Grains of glycogen are basically located here in the apical and basal portions of the receptor cells and in individual cells even around the nucleus. In the ampullae of the semicircular canals glycogen appears in the epithelium of the Planum semilunatum.

In the vestibular apparatus beside the receptor epithelium glycogen is also present in the nerve fibers ascending to the epithelium and further to the receptor cells. Among the nerve fibers it is possible to distinguish pale pink and bright red fibers. In the crista at the base of the hair cells, at the points of termination of nerve fibers on them, are seen bright red "drops" of glycogen. The otolith membrane and otoliths give a very bright positive SHIK reaction, but their staining is not connected with the presence of glycogen.

In a chick embryo of 14 days incubation nucleic acids are not distinguished from the preceding stage by their distribution and concentration. With the same protein reaction those cytological and cytochemical changes which took place at this stage in a receptor epithelium appear unusually clearly.

Most characteristic for this stage is the formation of contacts between the receptor cells and nerve fibers which have grown into the epithelium. In the sensory epithelium of the utricular macula the difference between supporting and receptor cells is sharply increased. All nuclei receptor cells lie at one level. They are larger, round, have a completely clear cytoplasm, small and quite pale clumps of chromatin and 1-2 very bright nucleoli, some of which are located eccentrically. The cytoplasm of receptor cells is significantly darker than of supporting cells. The cuticle is not pro-

nounced in them. The nuclei of supporting cells are small, oval and dark. The cytoplasm in the basal portion is very light, which indicates its property in protein and functional groups of protein molecules. The apical portions of supporting cells passing between receptor cells are dark and fibrillar. Sensory hairs give a very weak reaction to protein and its functional groups. /119

In the saccular macula the difference between receptor and supporting cells is less clearly pronounced. Sensory hairs give a more intensive reaction to protein and its functional groups. The sensory epithelium of the crista is characterized by the fact that in it the receptor cells located in one row, the round light nuclei of which are surrounded by light cytoplasm, stand out sharply. Between them are the apical portions of supporting cells, standing out clearly by their fibrillar cytoplasm, giving an intensive reaction to proteins and its functional groups. The contracted apical portions of these cells are expanded at the very surface forming characteristic triangles. These expansions attest to the contraction of the apical portions of receptor cells, i.e., to the differentiation of pitcher-shaped cells. Even the long sensory hairs of the crista give an intensive protein reaction.

The vestibular ganglion consists of large cells found in various stages of differentiation. A high concentration of protein and functional groups of protein molecules is characteristic both for the cytoplasm and for the chromatin of the neurons. Well defined processes of the ganglion cells give the same intensity of these reactions as the cytoplasm of the cell. In the nuclei of a majority of neurons there are two very bright nuclei. This same high concentration of protein is characteristic even for well developed cells of the capsule.

The 15th-16th days of incubation, when the differentiation of the vestibular apparatus of the inner ear of the chick embryo (which is visible under a light microscope) practically terminates, are of special interest.

The utricular and saccular macula are practically unchanged in comparison with the condition described for the 14th day of incubation.

The crista of both vertical semicircular canals attains almost complete development. On the middle of each crista there are two lateral outgrowths, the Septum cruciatum, and above the crista is seen an almost completely developed cupula, insofar as it is possible to determine this on fixed preparations. Receptor cells of the crista are elongated, and cylindrical or pitcher-shaped. Above the summit of the receptor cell a conusoid protrusion is visible, from the summit of which proceeds a long sensory hair entering the cupula for a certain distance. The cupula is striated longitudinally. Supporting cells have small oval darkly stained nuclei lying in 2-3 rows. Close by on the lateral wall is seen the differentiated Planum semilunatum,

consisting of light high cylindrical multinucleated epithelium with a very even surface. The small round nuclei of its cells are somewhat darker than the cytoplasm and are located usually in two rows.

Upon investigation of the utricular receptor epithelium of a chick embryo of 15 days incubation under an electron microscope first of all it is seen that even with small magnifications, the cytoplasm of receptor cells of the utricle sharply differs in electron density from the cytoplasm of supporting cells. Upon investigation of the general pattern of the structure of the receptor layer it is possible to easily establish a second characteristic of this stage: nerve fibers entering the receptor epithelium did not only grow up to the base of the receptor cells but even come into close synaptic contact with them (Fig. 33). The receptor epithelium terminates in a well-formed basal membrane (Fig. 33).

Investigations with large magnifications by an electron microscope indicate that the high electron density of the cytoplasm of a utricular receptor cell evidently is connected with the somewhat greater electron density of the matrix and its wealth in densely distributed elements of smooth membranes of the endoplasmic reticulum, most often forming narrow tubules (Figs. 34 and 35). In addition, in the cytoplasm there is observed a certain number of free ribosomes. In the apical surface of the receptor cell development of the cuticle in the form of a very small portion of amorphous or weakly granulated substance of high electron density begins, into which enter the axial root threads of the stereocilia. In the apical portion of receptor cells and also in the supernuclear and subnuclear zones a large number of round or oval small mitochondria are seen. The nucleus of the receptor cells of the utricle possesses significantly higher electron density than the nucleus of the surrounding supporting cells (Figs. 33 and 34). The oval nucleus is coated with a double membrane and filled with very densely distributed small grain material, which occupies the entire space of the nucleus. The matrix of the nucleus evidently is light, but due to the dense equal distribution of granule material is only seen with difficulty. The nucleoli by their density differ little from the substance of the nucleus and have very small dimensions. /120

The cytoplasm of the supporting cells of the utricle has a light matrix with richly granulated or rough membranes of the endoplasmic reticulum which form very large vesicles and cisterns in it. Significantly more rarely in it are found narrow tubules bordered by smooth membranes (Figs. 33-35). Moreover, the cytoplasm of supporting cells contains large number of mitochondria which are located mainly in the basal portion of the cell. The basal portion of the supporting cell is especially rich in granulated membranes of the endoplasmic reticulum in the shape of large cisterns located in a disorganized fashion in the cytoplasmatic matrix. Here the concentric distribution of membranes of the endoplasmic reticulum which is characteristic for this region of supporting cells of Corti's organ is never observed. In the basal portion is seen a well developed

basal membrane with a wide space between the two membranes: the internal one is a plasmatic membrane of the cell, the second is a basal membrane formed from a thickening of the fundamental substance of connective tissue. The basal membrane of the utricle may form a protrusion and penetrate rather deeply into the receptor epithelium of the utricle, repeating the path of the plasmatic membrane of the base of the supporting cells of the utricular macula.

The nucleus of the supporting cell has an oval or oval-round shape (Fig. 33). The basic substance filling it is completely transparent, and the small granules of high electron density are distributed in the basic substance rather loosely which makes the structure of the nucleus of the supporting cell lighter than that of a receptor cell. The cellular membrane is often thickened due to a dense layer of granules attached to the inner layer of the nucleus membrane. There is one (less often two) very small, moderately dense nucleolus consisting of accumulations of darker and lighter granules which, in the majority of cases, lie upon one another.

Between the cells of the receptor layer of the utricle are seen individual nerve fibers which have proceeded inside the epithelium. They pass between the bodies of supporting cells, reach the receptor cells and form synapses on their bases and on the lateral surface with the aid of individual nerve endings (Figs. 33-35). We did not succeed in finding the formation of nerve cups at this stage under the electron microscope, although under the light microscope individual scyphoid synapses are found in the receptor epithelium of the vestibular apparatus even at the stage of 12-th day of incubation. Investigation with greater magnifications of an electron microscope indicates that the nerve ending consists of neuroplasm covered with a clear single layer of plasmatic membrane. It possesses a completely light matrix and an insignificant number of small vesicles and single membranes and is completely deprived of fibrillar structures. In certain nerve endings individual small mitochondria are seen, but as a rule they are absent. In isolated portions it is possible to see the beginning of the formation of the presynaptic membrane at the base of a receptor cell. Most often two very thin and delicate plasmatic membranes, a cellular and a nerve ending lying very close to one another (Fig. 35), are seen.

It is necessary to note that at this stage it is difficult to say whether we are dealing with pitcher-shaped or cylindrical receptor cells, since the cellular shape is still poorly defined with an electron microphotograph. In fact, Figure 35 shows two light cells from the summits of which proceed sensory hairs, and it is possible to assume that in this case we are dealing with cylindrical receptor cells which are distinguished by the lighter cytoplasmic matrix. Their cytoplasm in the apical portion is distinguished from the cytoplasm of supporting cells by the absence of large cisterns formed by granulated (rough) membranes of the endoplasmic reticulum which are characteristic for cytoplasm of supporting cells of the utricle, but to say with assurance that this is actually so is very difficult.

On the summit of receptor cells there are bundles of sensory hairs each consisting of stereocilia and one kinocilium. The kinocilium has a well defined fibrillar apparatus of 9 paired peripheral fibrils and 2 single nonpaired fibrils surrounded by protoplasm with a light matrix in a very thin single layered external membrane appearing as a continuation of the protoplasmic membrane of the cell. The intercellular portion of the kinocilium is represented by a well-developed basal corpuscle. The stereocilia has the usual structure of thickened electron dense and finely granulated cytoplasm surrounded by a thin external membrane. As in the preceding stage an axial fibril is not seen in them.

With reactions for nucleic acids, the cytoplasm of both the receptor and supporting cells is still shown not to be homogeneous but to be fibrous granular. At the same time, at the apical ends of the cells a thin dark stria, possibly the beginning of the formation of the cuticle, is observed. The otoliths lying in the open space are completely colorless, which indicates a complete absence of nucleic acids in them (Fig. 37). The nuclear membrane and chromatin of the nucleus of the receptor cell gives a reaction of average intensity. The cytoplasm of these cells is distinguished by the higher concentration of nucleic acids in their apical portions than in the basal portions. The hairs have a weak nonspecific staining.

In the vestibular ganglion, the neurons composing it, both with reaction for nucleic acids and with protein reaction, reveal nonuniform concentration of these substances in different, often adjacent, cells. It is interesting that in addition, not only the intensity of the reaction of the cytoplasm but also that of the nuclear membrane (Fig. 38) changes.

The crista of the horizontal canal is of great interest not only by its shape but also by the cytological condition of its epithelium. There is still no cupula formed above it but the apical parts of the receptor epithelium covering it have the characteristic "unraveled" appearance. The impression is created that the apical ends of its cells are continued into the cytoplasmatic traces entering the endolymphatic cavity. Moreover, above the summit of the cells are seen plasmatic balls and clots of some kind of amorphous substance (Fig. 39). The receptor epithelium at the summit of this crista, as opposed to the slopes, is intensely vacuolized (Fig. 40). All of this is very similar to the descriptions given by Vilstrup (1950) and Savin (1960) of the developing cupula. However, like the latter author, we never observed here isolated and degenerate nuclei entering the protoplasmic traces forming a unique syncytium, although certain dark small inclusions are sometimes solved here. It is difficult to assume that the development of a cupula takes place differently in the cristae of the vertical and horizontal canal. It seems more probable that this process takes place more slowly in the crista of the horizontal canal which, in the chick embryo, is differentiated somewhat later than the crista of the vertical canals. On the 16-th day of incubation, above the crista of the horizontal

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canal, there is seen a small well-formed cupula and a subcupular space located under it. A receptor epithelium of the horizontal crista acquires a normal form towards the 19-th day of incubation (Fig. 41). At this stage both cristae of the vertical canals have a completely definitive structure. These stages can be practically considered as the end of differentiation of the vestibular portion of the labyrinth of the chick embryo, and the later periods of embryonal development evidently are basically connected with the complication of innervation and chiefly of synapsis, which cannot be completely investigated with the aid of a light microscope.

The basic process of morphological and cytochemical differentiation in subsequent stages are basically connected with the cochlear portion of the labyrinth. Those cytochemical changes which can be noted in the receptor formations of the vestibular portion are undoubtedly due to the influence of the beginning functioning of the organ.

On the 18-th - 19-th day of incubation the structures of the receptor epithelium of the maculae and cristae do not differ from those of a chick 5 days after birth and of adult individuals.

The receptor and supporting cells of the receptor epithelium of the crista differ sharply in the nature of their differentiation. The nuclei of supporting cells are small oval, with dark and very densely distributed clumps of chromatin, always located in the basal portion of the epithelium where they lie very close to one another. The parts of the cytoplasm containing nuclei are grainy and poor in RNA and protein. Above the part of supporting cells containing nuclei is located one layer of receptor cells rich in cytoplasm (Fig. 42). Concentration of protein and its functional groups is nonuniform both within the bounds of the cytoplasm of one receptor cell and in individual cells.

The nuclei of receptor cells are round and significantly larger than the nuclei of supporting cells. Their chromatin usually forms 2-3 large clumps and several small nuclei located along the periphery. Nucleoli in the amount of 1-2 have a bright pyronofile portion (RNA) and a dark peripheral ring of DNA. The nucleoli in the cells are often located eccentrically. The nuclei of receptor cells are located in 1-2 rows which are connected with the presence of two types of receptor cells, pitcher-shaped (Type I) and cylindrical (Type II) cells. In the pitcher-shaped cells the nuclei lie in the basal (expanded) portion; in the cylindrical cells they may be located closer to the apical end of the cell. This appears in all probability to be the cause of the destruction of the linear position of the nuclei of receptor cells which takes place at the later stages. In the receptor epithelium of the maculae and cristae we succeeded in observing 2-3 pitcher-shaped cells in one large scyphoid nerve ending.

The distribution of RNA in the cytoplasm of receptor cells

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changes very characteristically. While in the cylindrical cells RNA in the cytoplasm is distributed diffusely in the pitcher-shaped cells, the appearance of a bright, granular accumulation of RNA in the form of caps in the subnuclear, and sometimes simultaneously in the supernuclear part of the cell is observed. An accumulation of total protein and carboxyl groups of protein molecules (Fig. 42) is also observed here.

In the ampulla, the epithelium of the Planum semilunatum sharply differs from the receptor epithelium. Although the planum semilunatum also consists of high cylindrical multinucleated epithelium, very homogeneous oval small nuclei of these cells are located not in the basal but in the central portion of the epithelial layer. The basal part of the epithelium is completely free of nuclei and contains only protoplasmic parts of the cells. The apical surface of this layer is wavy due to the cupula-form nature of the extruding summits of the cells (Fig. 43).

The slopes of the cristae are covered with receptor epithelium, from which the nuclei are located in one basal row. Long hairs are seen on the surface of this epithelium.

The structure of receptor layers in the utricle macula in general is the same as in the crista, only their sensory hairs are shorter. The pitcher-shaped Type I cells are basically located in the central portion of the macula in the same lateral portions; between them lies the ever-increasing number of cylindrical Type I receptor cells. The margins of the crista are constructed of one row high cylindrical epithelium from which threads proceed, anchoring the otolith membrane.

Investigation of the structure of the utricular macula receptor epithelium of the chick before hatching indicates that at this stage the receptor epithelium of the utricular macula has already a more orderly form and the Type I receptor cells acquire the typical pitcher-shaped form. The bases of supporting cells lying on the basal membrane acquire a somewhat different shape. The latter is made more even. The apical surface of the receptor cell is occupied by a thick layer of cuticle from which proceeds a bundle of the already described sensory hairs (Fig. 44, 45). However on electron microphotographs we could not succeed in seeing real scyphoid synapses at this stage around Type I receptor cells, although individual portions of nerve fibers almost reach the cuticle.

The cytoplasm of the receptor cell is of higher electron density than the cytoplasm of a supporting cell. In addition, its matrix possesses a low electron density, and in it are located a great number of ribosomes, small vesicles and elements of smooth endoplasmic reticulum (Figs. 45 and 46). Directly under the cuticle are located large round and lighter mitochondria, descending along the apical portion of cytoplasm almost to the nucleus. Another group of smaller and more dense mitochondria is located in the subnuclear zone.

At the base of the receptor cell are seen the forming synapses, in which there are still no completely formed presynaptic membranes (Fig. 46), although in the presynaptic region of a receptor cell numerous synaptic vesicles are seen. A nerve fiber forming a synapse has a very light matrix, practically lacking electron density, in which there are located individual mitochondria and small vesicles.

A nucleus of a receptor cell bears great similarity to the nucleus of a supporting cell (Fig. 46), possessing a double nuclear membrane in which there are no well formed pores, and has the usual ultrafine structure with a well defined electron dense nucleolus of the correct round shape.

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The sensory hairs proceeding from the surface of the receptor cell usually consist of a large number of stereocilia and one kinocilium with the usual fibrillar structure. The stereocilia are constructed of electron dense osmiophilic thin fibrillar material, and in which there is a well-pronounced axial fibril.

Cytoplasm of supporting cells has a light, electron transparent matrix with an insignificant number of ribosomes and small vesicles located in it. Moreover, there are larger round formations surrounded by smooth, single-layer membranes, inside of which there is a certain content of an average degree of electron density. They are located chiefly in the apical portion of a supporting cell where they quite closely abut one another, but they are found even in the basal portions of the cytoplasm. They are similar to secretory granules. The nucleus of a supporting cell is very similar in structure to the nucleus of a receptor cell, differing from it by its somewhat higher electron density (Fig. 44).

In a 5-day old chick the cylindrical receptor cells have the same electron dense cytoplasm as the pitcher-shaped Type I receptor cells. Between them are seen very large supporting cells, the cytoplasm of which is filled with a large number of very large evidently secretory granules. Several of these granules are surrounded by a double membrane. The content of the granules has a somewhat higher electron density than the cytoplasmatic matrix, and this gives us the right to consider that these granules filling the cell are intravital formations and not the result of fixation. Moreover, similar granules but in a smaller number are found in supporting cells of the utricular macula at the hatching stage (Fig. 47b).

The cytoplasm of hair cells differs from the cytoplasm of supporting cells by its significantly higher electron density. It is thickly filled with elements of smooth and rough (granulated and agranulated) endoplasmatic reticulum in the shape of narrow tubules and cisterns of various profiles, vesicles and thin short protofibrils. In the cytoplasm is located a large number of dense mitochondria, primarily round in shape. The nucleus of receptor cells is covered with a dense double membrane and is filled with densely distributed small osmiophilic granules, which more or less equally fill

the entire space of the nucleus. In the nucleus there are from 1 to 3 very dense and small nucleoli (Fig. 47b). On the surface of the cell is a rather dense cuticular lamina from which proceed the stereocilia distributed both in simple and complex bundles (Fig. 47a). Small bud-shaped nerve endings abut the base of the cell; they are distinguished by the very light electron transparent matrix in which are located isolated synaptic vesicles and mitochondria (Figs. 47a, b). In the afferent nerve endings abutting the base of a receptor cell it is possible to see the attachment of mitochondria to the postsynaptic membrane (Fig. 47b).

In the cytoplasm of both receptor and supporting cells, Golgi's apparatus is extremely well developed and surrounded by secretory granules. It is noteworthy that in neighboring cells (receptor and supporting) the regions of Golgi's apparatus are mutually connected; i.e., they are located near the marginal cellular membranes, which the secretory granules approach closely. On an electron microphotograph the impression is created that the secretory granules of supporting cells are filled due to a secretion provided not only by their own Golgi's apparatus but also by a secretion of Golgi's apparatus of a neighboring receptor cell (Figs. 47c-e).

On this same electron microphotograph it is possible to see that a fully developed Golgi's apparatus is located in the nerve endings ascending to the receptor cell.

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The utricle of a 9-day old chick gives a more fully differentiated impression. Judging by the distribution of groups of sensory hairs, receptor cells lie in the central portions of the utricular macula in a very concentrated fashion, although each receptor cell, as before, is surrounded by supporting cells. Many receptor cells have the regular pitcher-shaped form; however, around none of them could we succeed in discovering under an electron microscope the indisputable and characteristic scyphoid nerve ending. Moreover, the number and mass of nerve endings around such receptor cells undoubtedly increases.

The nucleus of a receptor cell is located in the expanded part containing nucleus near the base. Smaller nuclei of supporting cells are located lower than the base of receptor cells near the basal membrane (Fig. 48). The shape of a supporting cell body on ultrathin sections is hard to define, since supporting cells are located between bodies of receptor cells and numerous nerve fibers growing into the epithelium. In contrast to the earlier stages, here a sharp difference between an electron density in the cytoplasm receptor and supporting cells is absent. The receptor epithelial layer is distinguished from the underlying connective tissue by the clearly defined and completely formed basal membrane.

The nuclei of receptor cells is somewhat larger than the nuclei of supporting cells and is distinguished from the latter by the normal round or round-oval form (Figs. 48 and 49). The nucleus is

covered with a clearly defined and well-formed double nuclear membrane; granules of peripheral chromatin densely abut this internal layer, and it is filled with more or less equally distributed small osmophilic electron dense granules. In the center of the nucleus is located a small and very compact nucleolus often having an irregular shape and consisting of a dense accumulation of the same osmophilic and electron dense granules as the chromatin of the nucleus (Fig. 48).

The cytoplasm of the receptor cell has a matrix of the same low electron density as the matrix of the cytoplasm of the supporting cell. On this light matrix in the receptor cell are located in a great number of elements of granulated and agranulated endoplasmatic reticulum forming narrow tubules, vesicles and cisterns of various profiles, small vesicles of the synaptic type, individual fibrillar structures and elements of Golgi's apparatus. Mitochondria in the pitcher-shaped receptor cells are located primarily in the apical portion (in the neck) of the cell. An especially high concentration of them is observed directly under the cuticle. Large accumulations are located also in the perinuclear zone and in the basal portion of the zone near the synapse. A similar distribution of mitochondria corresponds to their distribution in definitive Type I receptor cells and indicates a local specialization of individual portions of the cell (Fig. 48).

In individual receptor cells at this stage we first had to see how several mitochondria in the perinuclear zone closely approach the nuclear membrane and lie densely upon it, which evidently leads to an increase in the number of membranes on this local portion (Figs. 49, 50a and b). In rare cases it is possible to see destruction (dissolution) of the nuclear and mitochondrial membranes at the point of their contacts. The significance of this phenomenon remains incomprehensible to us. To date we have only succeeded in seeing a similar attachment of mitochondria leading to an increase in the number of membranes at the point of nerve fiber synapsis with the receptor cells of the utricle. This phenomenon takes place in the utricle of representatives of all classes of vertebrates and was first described by our laboratory group (Vinnikov, Gazenko, Titova, Bronstein et al., 1965). This phenomenon is increased with an elevation of the functional stress. Similarity of these patterns allows us to express the hypothesis that here, as in the synapse, the matter is one of energetic reinforcement of some chemical functional process unknown to us, connected directly with the nuclear membrane.

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Fibrillar structures visible in the cytoplasm of the receptor cell are not only scattered along the entire cytoplasm of the cell but also form individual accumulations in it.

As was already noted, at the apical end of the receptor cell is located a quite powerful and well developed cuticular lamina. It consists of a very dense amorphous or weakly granulated substance of average electron density. Within it are seen individual, more

osmiophilic vesicles, and under the surface itself lies a very thin osmiophilic electron dense layer, which is somewhat elevated at the point of attachment of the stereocilia. We could not succeed in finding any root structures of the stereocilia within the cuticular lamina.

Above the surface of the cuticle a bundle of sensory hairs is elevated, consisting of 50-60 stereocilia and one mobile kinocilium (Fig. 48). Stereocilia are covered with a more electron dense and osmiophilic plasmatic membrane, in comparison with the earlier stages, which appear to be a continuation of the plasmatic membrane of the cell. In the region of the lower margin of the cuticle, from the lateral surface of the cell a digitate outgrowth proceeds at first downward, and then upward, entering the neighboring supporting cell and delimited from its cytoplasm by a double plasmatic membrane consisting of external plasmatic membranes of the receptor and supporting cells. The upper surface of the digitate outgrowth is located under a desmosome which, at this stage, is growing inside the supporting cell and takes part in the formation of the reticular membrane (Figs. 51a, b).

Under the base and along the lateral surfaces of the receptor cell are seen numerous nerve endings, some of which are located as a large solid mass along the lateral walls of Type I receptor cells and reach their cuticle (Figs. 48, 53, 54). The neuroplasm of nerve endings is distinguished by very light "electron empty" matrix in which are located individual round mitochondria and a very insignificant number of elements of the endoplasmatic reticulum in synaptic vesicles. In individual nerve endings, attachment of mitochondria to the synaptic membrane, leading to an increase of its layers, is noted. Here there is often an accumulation of synaptic vesicles around the presynaptic membrane and a thickening of the membrane at the point of synapse. However the structure of the synapse still does not reach a definitive state everywhere.

The supporting cell as a rule has a body of irregular shape, and it is almost impossible to trace it totally on one electron microphotograph. The apical end of supporting cells does not have a cuticular lamina and is covered with an external plasmatic membrane, forming microvilli here. In one case we found a cylindrical rod with typical fibrillar structure on the surface of the supporting cell. However, its basal corpuscle did not fall into the section (Fig. 52). Along the sides of the summit of the supporting cell there are quite well developed electron dense osmiophil formations, i.e., desmosomes uniting individual cells. The substance of the desmosomes is expanded inside the supporting cell and gives a ring-shaped carcass inside it, thus forming elements of the reticular membrane (Figs. 51b, 52). /127

The apical end of the supporting cell is filled with numerous granules and covered by a single layered membrane, on the surface of which small ribosomes settle. The content of these granules has a

higher electron density than the plasmatic matrix surrounding them. These granules are found only in the form of accumulations located in the apical portion of the cell. They are not found in lower portions of the cellular body. The cytoplasm there contains individual elements of the endoplasmatic reticulum, Golgi's apparatus, lysosomes and single mitochondria (Figs. 48, 51a).

The nucleus of the supporting cell is somewhat smaller than the nuclei of receptor cells, and has a different, sometimes irregular shape covered with a dense double membrane. The space of the nucleus contains a light matrix and densely located small osmiophil granules with great electron density and from 1-3 nucleoli, larger than the nucleoli of receptor cells.

Thus a stage comparison between the data from histochemical and histological investigations of the histogenesis of the vestibular apparatus of the chicken in embryonal and postnatal periods of development and corresponding data from electron microscope investigation of the developing utricle (from the 4-th day of incubation to the 9-th day of postnatal life) shows that there exists a correlation between the cytochemical changes of a developing organ and the development of the submicroscopic structure of the cellular elements.

Conducting parallel investigations of the developing inner ear of the chicken under a light and electron microscope allowed us to somewhat shorten the exceptionally laborious electron microscopic investigations since thanks to them those difficult stages were determined, the electron microscope investigations being of the greatest interest, and the possibility of deciphering the changes taking place in the structure of receptor and supporting cells, as well as of investigating in detail the development of the perceiving and innervating apparatus, was realized.

Starting from the obtained data and hoping, if possible, to prevent repetitive description of several processes, proceeding equally with the development of the utricle and of Corti's organ, we permitted ourselves to deal in greater detail with the development of the innervation of the receptor cells with a description of the electron microscope investigation of the utricle, and to give a more detailed treatment of the developments of the proceeding apparatus, the stereocilia and the kinocilium with a description of the development of Corti's organ.

THE DEVELOPMENT OF CORTI'S ORGAN

The cochlear canal in the embryonal development of the chicken is first separated from the sacculus on the 7-th day of incubation. At this stage it is a short narrow blindly closed sac, at the anterior end of which is located the lagena. In the receptor epithelium of Corti's organ there is already noted a subdivision into receptor and supporting cells, which is manifested chiefly in the nature of cellular elements in the receptor layer. The future

receptor cells are located in the upper portion of the receptor layer and the supporting cells stretch from its free surface to the basal membrane. Their nucleus-containing portions are located below the bases of the receptor cells. Structurally the future receptor and supporting cells are not differentiated from one another under a light microscope. /128

Under small magnifications of an electron microscope the great polymorphism of the nuclei in the presence of individual nerve fibers passing between the cells is conspicuous in the receptor epithelium. Under great electron microscope magnification on the summits of many cells it is possible to see a kinocilium developing from the centrosome. At the same time, at this stage we did not once succeed in discovering the presence of formed kinocilia (Figs. 55, 56). The basal membrane is clearly pronounced for the entire length of Corti's organ. On the basal membranes the presence of a layer of completely transparent basic substance is noted which is located in separate portions between the basal membrane and the underlying mesenchymal cells. The presence of this substance, distinguished by a complete absence of electron density, forces one to assume that at this stage the beginning steps of the formation of the basal membrane take place. It is possible to connect this with the accumulation of mitochondria in the basal ends of the cells of the receptor layer in those portions of the cytoplasm of the cells which directly adjoin the basal membrane.

In the structure of the cytoplasm of the cells of the Macula communis of a 4-day chick embryo and of the cells of the receptor epithelium of Corti's organ of a 7-day embryo, in particular, in the structure of the cytoplasmic matrix and endoplasmatic reticulum, there are no differences. Just as in the cells of the Macula communis in a 4-day embryo, in the cytoplasm of the cells of Corti's organ on a 7-day incubation, free ribosomes predominate, which are not connected with membranes of the endoplasmatic reticulum.

Cytoplasm of the basal portion of the cell, located directly on the basal membrane, appears to be more differentiated if we take the degree of development of the endoplasmatic reticulum as an indicator of differentiation. In the cytoplasm of the basal portion of the cell, besides free ribosomes, there are already a significant number of membranes of the endoplasmatic reticulum, which, basically, are represented by cisterns of various shapes. Ribosomes on these, as before, are located rather loosely. Even elements of smooth endoplasmatic reticulum in the form of narrow caniculi are found in a small number. The cytoplasm of a cell is rich in mitochondria, which are basically concentrated in the apical and basal portions of the cell; mitochondria, as a rule, have a round or oval shape and a light matrix, and densely distributed, not always uniformly oriented, cristae (Fig. 57). Rod-shaped mitochondria are absent here.

As was already noted, on the summit of many cells are seen the developing kinocilia (Figs. 56, 58), with the supporting structure

of 9 pairs of peripheral and two central fibrils which is characteristic for them. In longitudinal section the central fibrils are not easily visible in the kinocilia, but they are clearly traced in the basal corpuscle which is connected with the kinocilium and from which, evidently, the kinocilium develops (Figs. 58, 60). Beside the basal corpuscle, at an angle to it, is located the centriole from which fibrils proceed (Fig. 58). The location of fibrils is especially visible in cross section. On all the electron microphotographs it is possible to clearly detect that the endoplasmatic membrane of a cell directly turns into a kinocilium and forms an external membrane upon it.

Sometimes from a kinocilium, more precisely from its basal corpuscle, stretch certain "threads" or "tubules" which under great magnification are similar to the canaliculi of the smooth endoplasmatic reticulum. It is completely possible that they are not connected with the basal corpuscle of the kinocilia, and the correspondence of their spatial orientation is purely accidental. This cell with the developing kinocilia, in which are found the above-described tubules of smooth endoplasmatic reticulum, is of somewhat higher electron density which, as we will see in the later stages, is characteristic for differentiated receptor cells of Corti's organ of birds. /129

On the summit of individual cells it is possible also to see the beginning stages of development of kinocilium (Fig. 59). They are formed by means of a protrusion or formation of an outgrowth of cytoplasm. The electron density of these is higher than the electron density of the cytoplasm of a cell. Indeed, in some cells it is possible to see a very thin layer of cytoplasm of the same high electron density around the external plasmatic membrane. Inside the forming stereocilium the cytoplasm has a fine fibrous or fine granular nature; i.e., the same structure which is characteristic of stereocilia of definitive cells. The plasmatic membrane of a cell is directly continued onto the stereocilia forming its external membrane. We did not succeed in discovering an axial thread in such developing stereocilia.

As was already mentioned, the nuclei of the receptor epithelium of a 7-day old chick embryo are distinguished by their great polymorphism. However, the structure of the nucleus does not essentially change. The double-contoured nature of the nuclear membrane is made clearer due to an increase in the intervals between membranes, and the presence of pores in the nuclear membrane (Fig. 55) becomes more apparent.

This rudiment of receptor epithelium gives an intensive reaction for nucleic acids (DNA and RNA), and also for total protein and functional groups of protein molecules.

The rudiment of the cochlear ganglion at this stage consists of small cells, the nuclei of which are provided with bright nucleoli.

The comparatively small rim of cytoplasm around the nucleus is distinguished by a rich content of nucleic acids, proteins and functional groups of protein molecules.

On the 8th-9th day of incubation a large number of nerve fibers grow up to the rudiment of the receptor epithelium, which fibers may be well impregnated with silver. These fibers not only grow inside the epithelium but also individual ones reach the base of the future receptor cells. In the apical portion of receptor epithelium there is a large number of cells in various stages of mytosis. On the posterior end of the cochlear canal the backing of the tegmentum vasculosum is formed.

The cytochemical characteristics of this receptor epithelium are not distinguished from those already mentioned at this stage for the receptor epithelium of the utricular macula. An unusual richness in nucleic acids (both DNA and RNA) is characteristic of the receptor epithelium of all rudiments of receptor formations of the inner ear. However, toward the end of the 9-th day the concentration of nucleic acid in cells of the rudiment of the receptor epithelium of Corti's organ falls, while the reaction for protein decreases in the basal portions of the receptor epithelium and increases in the apical portions. The reaction to functional groups of protein molecules does not change. In the cochlear ganglion the level of concentration of nucleic acids, as well as that of total protein and functional groups of protein molecules, is preserved.

Toward the 10-th day of incubation the nerve fibers have already reached the level of the base of the future receptor cells. The tegmentum vasculosum is developed along the entire length of the cochlear canal which continues to increase in length and forms simple smooth folds which hang in the lumen of the cochlear canal in the form of a sparse, smooth fringe. The cells composing them have a round shape and are densely distributed. Traces of dark cells are seen among them.

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The medial wall of the cochlear canal consists of a dense row of high cylindrical cells. A dark stria covers them from the top and below it is located a yellow homogeneous stria. It is possible that this is the backing of the tectorial membrane. Nerve fibers approach the cells forming a plexus under their base, the Plexus externus. A blood vessel passes along the base of Corti's organ from the side of the ganglion. Differentiation of the cochlear canal of birds does not take place simultaneously along the entire length: it begins from the posterior end, i.e. from the base, and moves toward the anterior end, i.e. the summit. At this stage the anterior end of the cochlear canal has an almost indifferent nature. Nerve processes which are well impregnated with silver are seen in the cochlear ganglion. The peripheral processes of the ganglionic cells grow to the rudiments of the sensory epithelium and enter inside it, and the central processes during 9-11 days of incubation grow to the brain and unite with it. The nerve fibers inside the

receptor epithelium are still more intensively attached and entwine the bases of the receptor cells. This process gradually moves forward to the summit of the cochlear canal. In the cochlear ganglion the cells are significantly smaller than in the vestibular ganglion.

The receptor epithelium of the Papilla acustica basilaris of a chick embryo of 11 days incubation, upon investigation with an electron microscope, is interesting due to the fact that at this stage the divergence between the differentiation of the receptor epithelium of the summit and base of the cochlear canal appears to be unusually clear.

The rudiment of the receptor epithelium, little differentiated from that which was described by us for the 7-th day of incubation, is seen on a section through the summit of the cochlear canal (Corti's organ). Cells of this epithelium still are of indifferent structure. On the summit of individual cells it is possible to see the initial steps of the development of the sensory hairs. Indeed, as opposed to the 7-day stage, here several differences in the structure of the cytoplasm are noted. Thus in several cells the supernuclear portion of the cytoplasm is rich in dense mitochondria, while other mitochondria are present in a very insignificant number, and the cytoplasm of the supernuclear portion is filled with large, light brown granules covered with a single membrane. If we proceed from the structure of a definitive receptor epithelium, the first cells are converted into receptor cells and the second into supporting cells. The nuclei of those and of other cells are little differentiated from those described for the receptor epithelium of the 7-day old embryo, and we could not succeed in discerning any clear dimorphism between them.

The underlying cells of the mesenchyma adjoin the basal membrane rather closely, which appears to be well formed and consists of two clearly defined layers. Evidently only the initial stages of development of the basilar membrane take place here.

We observe a completely new picture on a section through the base of Corti's organ. Here there is seen an already completely clear division of cellular elements into receptor and supporting cells. The first (receptors) have a well defined low cylindrical shape; their summits are enveloped by desmosomes and seemingly form a "little sleeve" contracted at the base. The region of this "little sleeve" evidently corresponds to the future cuticle and is filled, basically, with a granular substance. The small granules are thickened at the summit of the cell under the plasmatic membrane at the point of origin of the stereocilia. The stereocilia are in the form of very uniformly distributed outgrowths of the external plasmatic membrane, and inside they contain the same but even denser, /131 finely granulated substance. The cytoplasmatic matrix of a receptor cell is little different in its electron density from that of a supporting cell. However the cytoplasm of a receptor cell is significantly more dense than the cytoplasm of a supporting cell, due

to the large number of mitochondria, vesicles, rough and smooth membranes of the endoplasmatic reticulum forming vesicles, tubules, cisterns of various profiles and individual ribosomes. Golgi's apparatus is well developed and is located in the subnuclear part of a receptor cell. The nucleus of a receptor cell is round, large and light. It is covered with well-developed double nuclear membrane in which it is possible to distinguish thickened portions and pores. The internal content of the nucleus (chromatin) is represented by a rather loosely distributed fine granular substance (Fig. 60).

The cytoplasm of a supporting cell has a significantly lower electron density than the cytoplasm of a receptor cell. With an equally low electron density of the matrix, this low density of the cytoplasm is explained by the fact that the cytoplasm of the supporting cell is poorer in various cytoplasmatic structures and organelles than the cytoplasm of a receptor cell. Granules of average size are basically present in the cytoplasm of supporting cells covered with single membranes, the contents of which in individual granules differ little in electron density from the cytoplasmatic nature. The mitochondria in the cytoplasm of the supporting cell are found in an insignificant number. The nucleus of a supporting cell is distinguished by its oval shape, more dense location of granules and the presence of 1-2 dense small nucleoli. The mitochondria in the supporting cell are concentrated in the subnuclear zone around the basal membrane, which evidently is connected with the processes of formation of the basilar membrane.

The process of formation of the basilar membrane is significantly advanced. Between the basal membrane and the underlying endothelial (mesenchymal) cells is formed a layer of the basic substance of the basilar membrane, completely lacking electron density. The formation of protofibrils directed perpendicular to the long axis of the cochlear canal takes place in this layer. The process of formation of fibrillar bundles (Fig. 61) begins. On the 11-th day of incubation in the receptor epithelium of Corti's organ on preparations treated for nucleic acids are seen very clear, completely transparent nuclei of the receptor cells. Below lie the smaller and darker nuclei of supporting cells. The cytoplasm has a fibrous nature. The intensity of a reaction for RNA is somewhat increased in the apical part of the cell. Cellular borders are almost not visible. In the apical part of sensory epithelium a large number of mitosis is seen.

The cochlear ganglion gives a more intensive reaction for nucleic acids. The future neurons are distinguished by smaller nuclei and in the vestibular ganglion. Rather dark thin fibers proceeding from the neurons are clearly visible. Their cytoplasm is stained somewhat darker than the cytoplasm of cells of the receptor epithelium which indicates the high concentration of RNA in them.

On the 12-th day of incubation the pattern of distribution of nucleic acids and the structure of the receptor epithelium of Corti's

organ changes somewhat. The nuclei lying in the apical part of the receptor epithelium and belonging evidently to the future receptor cells are rounder and lighter. In many places in the apical zone of the receptor layer are seen the daughter cells which have just dispersed after mitosis, and which are at various stages of formation. The chromatin in the nucleus of the receptor cell of Corti's organ /132 is distributed equally along the entire nucleus forming 2-3 large clumps and several small clumps. In the receptor epithelium cell contours are not seen with this reaction. With a reaction for total protein a monotypical staining is observed. Both the protein and its functional groups reveal a quite low concentration in the receptor epithelium of Corti's organ at this stage. Above the sensory epithelium of Corti's organ is seen the tegumentum vasculosum, as before, forming regular folds. The nuclei of the cells composing it are round with small very densely distributed clumps of chromatin. The contours of the nucleus are seen very clearly. Cytoplasm surrounding them is clotted and fibrous. The cytoplasmatic cellular membrane can barely be seen and the contours of the cell cannot be traced. Long cylindrical cells with a round apical end compose the peripheral portion of each fold. The surface of the edge of the tegumentum vasculosum is irregular and "webbed". Upon reaction for nucleic acids, total protein and carboxyl groups of protein molecules its cells have almost a monotonal staining. Inside each fold is a thin (narrow) but clearly pronounced lumen into which interindividual connective tissue cells and blood capillaries enter. Upon treating the preparation for DNA according to Felgen's method, the nuclei of the cells of the tegumentum vasculosum produce the impression of reticulated cells. At the same time the cytoplasm is completely colorless. Upon reaction for carboxyl groups the borders of the cells stand out clearly. The cytoplasm has a light background and irregularly located small sinuous fibers.

The cells of the cochlear ganglion still have an embryonal character. Upon reaction for nucleic acid, neurons of the cochlear ganglion are distinguished by the almost completely colorless large nuclei and small cellular bodies. The contours of the nuclei (nuclear membrane) are practically colorless. The small nuclei which are present here give different degrees of intensity of reaction for nucleic acids. The contours of the cellular body are not seen. With a reaction for total proteins small oval nuclei seem to have a reticulated structure. Their cytoplasm gives a more intensive reaction than the cytoplasm of ganglionic cells. Carboxyl groups which are especially richly represented in the nucleolus and the cytoplasm of the cell have a high concentration in the cells of the cochlear ganglion.

The lagena macula lying at the blind end of the cochlear canal (Papilla acustica lagena) of the sensory epithelium has the structure which is usual for macula. It consists of a multiple high cylindrical epithelium, along the apical edge of which are located lighter round distinct nuclei. Small grains of chromatin in them are close to the nuclear membrane. The nuclei of supporting cells

are located in the basal part of the epithelial layer and have an oval shape. At this stage sensory hairs are still not visible under a light microscope. The cytoplasm of the cells is intensely vacuolized. The nuclei are oval and quite small. The chromatin is characterized by average concentration of DNA and there is a weakly positive reaction even in the karyoplasm. The nuclear membrane is not very clearly visible. The cytoplasm has a higher concentration in the apical portion. In the basal parts of the epithelium the cytoplasm acquires the nature of residual impressions.

On the 13-th day of incubation in the posterior portions of the cochlear canals under the sensory epithelium of Corti's organ appears a very thin but well-defined tectorial membrane. In the receptor epithelium of the cochlear canal glycogen is not observed before the 13-th day of incubation. A positive reaction for glycogen appeared at this stage from the tegumentum vasculosum, not in the form of granular inclusions but rather in the form of weakly diffuse stain. The very bright grains of glycogen are seen in the nerve fibers ascending to the receptor epithelium. The tectorial membrane gives a very bright positive SHIK reaction, but it does not depend on the glycogen, and we still have not been able to establish its nature. /133

Neurons of the cochlear ganglion have a pink diffuse tint. However, under the action of saliva this tint is not completely removed; consequently it does not depend only upon glycogen.

On the 14-th day of incubation with Felgen's reaction for DNA there first appears a division of the cells of the tegumentum vasculosum into two types. They are distinguished by the degree of concentration of DNA and a pale or bright contour of the nucleus.

With a reaction for carboxyl groups, the changes which have taken place in the receptor epithelium of Corti's organ and the lagena macula appear very clearly.

In the receptor epithelium of Corti's organ, receptor cells form a distinct row. Supporting cells are seen in places between them. A receptor cell has a round light nucleus, is poor in chromatin, with a very bright, centrally located nucleolus. The nucleus of a receptor cell is poor in DNA, total protein and functional groups of protein molecules. The nucleolus, on the other hand, contains a great concentration of these substances. In the center of the nucleolus there is also RNA in a fairly high concentration. The cytoplasm of receptor cells is significantly richer in carboxyl groups, RNA and total protein than the cytoplasm of supporting cells. On the summit of receptor cells certain dark formations are seen; evidently the cuticle and short sensory hairs which are beginning to be formed.

Supporting cells lying under them have smaller oval nuclei and a bright fibrous cytoplasm. Their supernuclear portion has the nature of narrow fiber traces, which cannot always be traced along the

surface of the epithelium on a thin section. Under them lies a clearly defined basal membrane possessing a high concentration of total protein and carboxyl groups of protein molecules. Endothelial cells with elongated light nuclei adjoin them. Cytochemically, as was already noted, a supporting cell is distinguished from a receptor cell by a significantly lower concentration of RNA, total protein and functional groups of protein molecules in its cytoplasm. The nucleus of a supporting cell has approximately the same cytochemical characteristics as the nucleus of the receptor cell.

In the cells of the tegumentum vasculosum of a 14-day old embryo, upon reaction for nucleic acids, total protein and functional groups of protein molecules, the nuclei have the same differences which were described with Felgen's reaction for DNA. Nuclei with a delicate nuclear membrane poor in these substances and in chromatin are located primarily in the central portion of the poles; the bright nuclei, the nuclear membrane and chromatin of which have a high concentration of the enumerated substances belong, as a rule, to the peripheral cells. In certain cells the plasmatic membrane stands out more clearly. The cells are located densely together and their cytoplasm contains approximately the same concentration of the substances.

In the cochlear ganglion differentiation of the cells most sharply appears in the change of their nuclei. The nucleus of a future neuron is larger and vesiculiform. Its cellular body has an irregular shape, and from it proceeds a central and peripheral process but on a section it is rarely possible to see both processes on one and the same cell. The highest concentration of total protein, nucleic acids and functional groups of protein molecules is observed in the nucleolus and in the nuclear membrane. The chromatin of a nucleus is rather poor in these substances. In the cytoplasm the concentration of RNA, total protein and functional groups is still moderate, but in individual neurons it begins to be elevated with the preservation of their equal distribution in the cytoplasm. Among the nerve fibers which are distributed in bundles are seen the extended nuclei of Schwann's glia with an average concentration of total protein and functional groups of protein molecules in their nuclear membrane and in the chromatin which contains even DNA in a significant concentration. /134

As was already noted above, the 15-th day of incubation is of special interest in the investigation of the receptor epithelium of Corti's organ, since under a light microscope receptor cells of Corti's organ are completely differentiated at this stage.

If the receptor epithelium of Corti's organ at this stage is compared under a light microscope with receptor epithelium of the utricular macula, then it is possible to find much in common in the basic features of the structure of their receptor cells. Just as in the utricular macula, the receptor cells of Corti's organ are distinguished from the supporting cells by the high electron density

of their cytoplasm, although to a somewhat smaller degree than in the first. On the other hand, in the receptor epithelium there are the reverse relations between the nuclei of receptor and supporting cells. While in the utricular macula receptor cells are distinguished by the higher electron density of their nuclei, in the receptor epithelium of Corti's organ the nuclei of receptor cells are significantly lighter than the nuclei of supporting cells. Both on the surface of receptor cells of the utricle and on receptor cells of Corti's organ are seen bundles of sensory hairs consisting of a great number of stereocilia and one kinocilium of the characteristic fibrillar structure in each bundle. Just as in the receptor epithelium of the utricle, within the receptor epithelium of Corti's organ are seen numerous nerve fibers passing between the bodies of receptor cells and forming synapses with the aid of small nerve endings on the base of receptor cells of Corti's organ (Fig. 62). Both organs of the receptor epithelium terminate in a well-developed basal membrane, which passes parallel to the plasmatic membrane of the base of the supporting cells. However, while under the basal membrane of the utricle a connective tissue is located, in Corti's organ between the connective tissue of the basal membrane a forming basilar membrane (Fig. 63) is located.

The cytoplasm of a receptor cell of Corti's organ in a chick of 15 days incubation has a completely light matrix in which are located rough (granular) membranes of the endoplasmic reticulum in the form of larger vesicles of various profiles and dimensions. In the perinuclear regions there are even found elements of smooth endoplasmatic reticulum in the form of narrow tubules located in parallel; possibly these are elements of the developing Golgi's apparatus. Between them in the cytoplasmatic matrix is found even a certain number of free ribosomes. The round or round-oval mitochondria lie basically in the apical and subnuclear part of the cytoplasm, and in the apical part there are found round, more dense, and small mitochondria, but in the subnuclear part only large mitochondria are found. On the apical surface of the receptor cell, under the plasmatic membrane itself, the cuticle begins to develop in the form of a very small portion of amorphous or weakly granulated substance of high electron density.

The nucleus of a receptor cell of Corti's organ has a round shape. The double contoured membrane covering it is very thin and delicate. Small granules filling its entire area are distributed very loosely, due to which the nuclei of receptor cells appear to be lighter under the electron microscope than the nuclei surrounding the supporting cells. The nucleolus, which is comparatively small, dense and well outlined in an ultrafine section, is not always found in each receptor cell (Fig. 62). /135

On the summit of a receptor cell of Corti's organ is located a bundle of sensory hairs which, just as in a receptor cell of the utricle, contains many stereocilia and one kinocilium with the characteristic fibrillar structure (Fig. 64). In this figure it is

possible to see that in an individual stereocilia the axial fibril begins to develop in its central part. The presence of a kinocilium in the avian auditory receptor cell has a very important significance, insofar as in the mammalian receptor cells of Corti's organ the kinocilia is absent and only a basal corpuscle remains of it.

Nerve fibers approaching the base of the receptor cells of Corti's organ form numerous synapses which, however, still do not have a completely developed synaptic structure at the base of the receptor cell. Upon investigation of these synapses under great magnification of an electron microscope the question can only be one of the small thickening of the presynaptic membrane. The matrix of the synaptic ending is very light and contains single mitochondria and membranous structures.

The cytoplasm of a supporting cell of Corti's organ is of lower electron density than the cytoplasm of a receptor cell. The presence of a rather large number of vesicles and cisterns of granulated (rough) membranes of the endoplasmatic reticulum, the largest of which have some kind of substance of average electron density inside them, is characteristic of the apical part of a supporting cell. There are practically no mitochondria in this part of the cytoplasm. On the apical surface of these cells there is a certain number of microvilli. This apical part of a supporting cell is usually located between the summits of the two neighboring receptor cells and, gradually narrowing, is converted into a narrow cytoplasmatic trace passing between the bodies of the receptor cells. Below the base of the receptor cells, the body of the supporting cell is expanded and turns into the expanded portion containing the nucleus, usually rich in small, round dense mitochondria. Besides the mitochondria in this part are found numerous membranes of the endoplasmatic reticulum, most frequently of the type of granulated (rough) membranes, bounding rather broad rimal spaces, either forming cisterns or large vesicles of various shapes. All these formations are filled with internal contents of average electron density. In the region around the basal membrane a concentric distribution of membranes of the endoplasmatic reticulum around the nucleus is observed, and in those cells whose nucleus is located higher, the same concentric location of membranes of the endoplasmatic reticulum is observed in the subnuclear regions in portions directly situated on the basal membrane. Numerous chiefly round mitochondria are found among them (Figs. 62, 65a).

The basal membrane is double, divided by a very smooth narrow fissure. The internal membrane is a plasmatic membrane of the supporting cell and the external is the real basal membrane around which is seen a fibrillar thickening of the fundamental substance of the connective tissue.

The nucleus of a receptor cell is of oval or oval-round shape with a double nuclear membrane, somewhat reinforced from the internal side by the granular nuclear substance which closely adjoins it. The granular substance filling the space of the nucleus of a support-

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ing cell is of higher electron density and is more densely distributed in comparison with the nucleus of the receptor cell. The nucleoli (one or two) are very compact, of small dimensions and are distinguished by high electron density.

The developing basilar membrane closely adjoins the basal membrane from below; a thickening of thin bundles or individual fibers directly adjoins the basal membrane. The basic or intermediate substance of the basilar membrane is electron-transparent and completely structureless, since even under very great magnification of an electron microscope it produces an impression of emptiness (Figs. 63, 65a and b). However, in this amorphous substance, parallel to the basilar membrane, there are bundles of very thin protofibrils of high electron density, separated from one another by layers of this same amorphous substance. As it is possible to see in Figures 63 and 65a and b, the number of thick and thin bundles of protofibrils varies in different portions of the basilar membrane.

From the side of the Scala tympani the basilar membrane is in very close contact with the lining cells, but, as opposed to the basal membrane, the protoplasmic membrane of the cells is in direct contact with the amorphous substance of the basilar membrane (Figs. 65a and b).

The tectorial membrane, under an electron microscope, has the form of a pale, amorphous or weakly granulated substance and possesses a low electron density. In Figure 2 it is possible to see how the substance of the membrane forms free helmet-like spaces above the bundles of sensory hairs, ascending directly to the surface of supporting cells, since the microvilli of the latter prove to be directly immersed in the substance of the tectorial membrane.

The cytological structure of the receptor epithelium of Corti's organ on the 15th day of incubation is very interesting under a light microscope; here it is easy to trace, not only after fixation by Champi's method and staining with ferric hemotoxalin, but also on preparations treated histochemically for nucleic acids, proteins and especially for carboxyl groups of protein molecules. On a cross section of Corti's organ of a chick embryo of 15 days incubation, it is seen that the receptor cells form a very even row along the external edge of the receptor epithelium. Among them pass the apical parts of supporting cells having the form of traces. However, among the supporting cells, the receptor cells are not quite uniformly distributed; they are found rather in groups of various sizes. Nearer the anterior hyaline cells which, at this stage, have already taken on their characteristic appearance, groups of receptor cells are more numerous and are more densely arranged. In the direction toward the posterior hyaline cells the size of such groups and the density of their distribution decreases. The parts of supporting cells containing the nuclei lie under the receptor cells and their nuclei, located within the epithelial layer in a rather disorderly fashion,

form several irregular rows. A receptor cell has a low cylindrical shape with a somewhat narrowed base, dark cytoplasm and a light round nucleus containing quite numerous small clumps of chromatin and a dark nucleus. On the summit of a receptor cell is seen a dark band of cuticle above which rises the sensory hairs. Above the ends of the sensory hairs lies the dark coarsely fibrous tectorial membrane, which is elevated above the hairs in the region of the anterior hyaline cells and then fits close to the summits of the sensory hairs. The nucleus of the supporting cells is oval and smaller than the nucleus of the receptor cells. It seems darker due to the large number of dark chromatin grains. The cytoplasm of the supporting cell is significantly lighter than the cytoplasm of the receptor cell. /13

Pyroninophiles are not visible with reactions for nucleic acids in the nucleoli of receptor cells; with a reaction according to Brachet's method they are stained in a bright green color. The chromatin is pale, which indicates its low DNA concentration.

Reaction for total protein is especially intensive in the apical parts of the receptor cell cytoplasm, and in the tectorial membrane. The contours of the nuclei of receptor cells are darker than the contours of supporting cells. The cellular membrane of receptor cells is readily visible, although it contains an average concentration of protein. The cytoplasm of supporting cells and of the basal parts of receptor cells has the same concentration of protein. A significantly more intensive reaction is observed in the connective tissue cells lying below the basal membrane. The latter do not completely fit the basal membrane closely, but even with the aid of a light microscope it is difficult to say whether this is connected with the beginning of the development of the basilar membrane. Carboxyl groups of protein molecules, as well as total protein and a small concentration of protein, are revealed in the apical portions of receptor cells and in the tectorial membrane. A somewhat lower concentration is observed in the nuclear membranes of receptor and supporting cells and in the nucleolus of receptor cells. The chromatin of the nuclei of receptor cells, the nucleoli and the cytoplasm of supporting cells have an average concentration of protein. A weaker concentration is observed in the hairs. Thus, the distribution of total protein in carboxyl groups of protein molecules does not completely correspond, and differs in a number of details. The small intensity of reaction in the apical parts of the cytoplasm of receptor cells and in the tectorial membrane is general and invariable for these reactions.

At the anterior end of the cochlear canal, which continues to grow, the formation of new folds of the tegumentum vasculosum is observed. They are laid down in the form of a dense round accumulation of cells with an even, round, external edge. Then inside such an accumulation is formed the beginning of a narrow, then broad, lumen into which grow the capillaries and cells of connective tissue. The configuration of the folds of the tegumentum vasculosum changes at this stage; they become round with a broader lumen and begin to differ

in length. But even part of the former narrow longer folds with a narrow lumen remains. Cytological and cytochemical changes in the tegumentum vasculosum are not noted at this stage; moreover, a certain increase in the reaction for DNA and RNA, total protein and carboxyl groups of protein molecules is observed.

The cochlear ganglion does not noticeably change in comparison with preceding stages. The concentration of nucleic acids, total protein and carboxyl groups of protein molecules is significantly lower in it than in the vestibular ganglion.

Toward the 18th-19th day of incubation, the avian Corti's organ begins to acquire its definitive form. Anatomically all the parts of Corti's organ first become well developed in the posterior parts of the cochlear canal: anterior homogeneous cells, anterior hyaline cells proceeding from the anterior homogeneous cells, the tectorial membrane and the receptor epithelium itself and the nerve fibers growing into it. It is difficult to draw an incontrovertible conclusion with the aid of a light microscope in regard to the condition and degree of maturity of the synapse. In the structure of the anterior homogeneous and anterior hyaline cells, it is still not possible to note any clear difference. However the portion of the epithelium corresponding to homogeneous cells is represented by a double-rowed high cylindrical epithelium, which turns into a one row (one-layered) high cylindrical epithelium of the hyaline cells. The tectorial membrane already covers the entire length of the sensory epithelium of Corti's organ but does not touch its sensory hairs everywhere. It is completely possible that this is the result of fixation. Receptor cells, as before, form one layer of cylindrical elements, the height of which varies. The highest cells are located in the anterior parts of the papilla. The cytoplasm of receptor cells differs sharply from the cytoplasm of supporting cells. The base of the receptor cell is round, sometimes somewhat elongated in the middle, evidently due to the approaching nerve fiber. The nuclei of supporting cells are located below the base of the receptor cell in 2-3 irregular rows. Their cytoplasm, as before, is very light. Tonofibrils are not seen in supporting cells. /138

Nucleic acids reveal a greater intensity of reaction for RNA in the cytoplasm of receptor cells than in supporting cells. The intensity of reaction for DNA is the same both in nuclei of the receptor and in nuclei of supporting cells.

With reaction for total protein the sensory epithelium of Corti's organ gives a reaction of average intensity. Receptor cells have a greater concentration of protein than supporting cells. The sensory hairs are somewhat lighter than the protoplasm of receptor cells. The tectorial membrane gives the most intensive reaction. With a reaction for carboxyl groups of protein molecules, the nuclear membrane of receptor cells gives a very intensive reaction since the nucleus appears to be stained along the periphery. Nuclei of supporting cells are lighter, as is their membrane. The chromatin in

them forms sparsely distributed clumps having an average concentration of carboxyl groups. Cytoplasm of supporting cells, as with other reactions, is much lighter than the cytoplasm of receptor cells, which indicates a weak concentration of protein and its functional groups in them.

Folds of the tegumentum vasculosum grow in length. Their internal lumen greatly decreases, and toward the end of the 16th day of incubation in the posterior part of the cochlear canal they hang in its cavity in the form of a long even fringe. A subdivision of the cells into light and dark cells appears in their epithelium, which appears specially sharply on preparations treated for total protein and its functional groups. The subdivision of the epithelial cells into light and dark begins in the posterior part of the cochlear canal and gradually spreads toward its summit (toward the lagena). With a reaction for carboxyl groups their intensity is significantly higher in the more differentiated portions of the tegumentum vasculosum at the posterior end of the cochlear canal. Here there are also light and dark cells but they are less sharply differentiated from one another in concentration of carboxyl groups than takes place with the reaction for total protein. With the appearance of nucleic acids the differences between the light and dark cells are manifested not only in the intensity of reactions for RNA but also in the structure of the nuclei in the concentration of DNA of their chromatin.

Toward the 19-th day of incubation processes of differentiation are strengthened in the cochlear ganglion. Neurons of the cochlear ganglion, as before, exceed cells of the vestibular ganglion in size. "Erosion" of the nucleoli appears in their nuclei on the 17th-18th day of incubation. Nucleoli of a majority of neurons are distinguished by the fact that traces appear in them directed from the center of the nucleolus to the periphery of the nucleus where accumulations of chromatin are located. This is especially clear with reaction for carboxyl groups of protein molecules. At the same time the cytoplasm of a neuron is dark but the carboxyl groups in it are distributed irregularly: the highest concentration of them is observed in the perinuclear zone and is significantly smaller on the periphery of the cell. Toward the 19th day of incubation the cochlear ganglion at the base of the cochlear canal approaches its definitive structure. Cell-satellites are distinguished by a very weak reaction for carboxyl groups and are barely noticeable among the significantly darker neurons. In neurons of the cochlear ganglion a very high concentration both of DNA and RNA is observed. However with the reaction to total protein its intensity is rather weak. The nucleoli at the same time are stained more darkly but those "traces" which were observed on the 17th-18th day of incubation with a reaction to carboxyl groups are not seen. /139

Beginning with the 17th day of incubation glycogen in the sensory epithelium begins to decrease, and toward the 2nd-5th day of postnatal life glycogen in the receptor epithelium is not revealed

histochemically. At this time it is found only in the epithelium of the planum semilunatum of the ampullar cristae, and a very insignificant amount of it is found in the marginal portions of the tegumentum vasculosum.

The changes in the sensory epithelium of Corti's organ visible under a light microscope are practically terminated. Directly after pecking out there is observed a rather strong increase in the intensity of reaction for RNA, total protein and functional groups of protein molecules of the receptor cells of Corti's organ (Fig. 66). Later the intensity of these reactions noticeably decreases. However several changes in the concentration of these substances observed after pecking out can be associated with not only the process of differentiation but also the influence of the specific function of the receptor cells which has begun. In the given case it is difficult to judge this, since the connection between specific functioning of Corti's organ and the general exchange cycles in Corti's organ of birds has not been investigated to date. Differentiation of the entire receptor epithelium of Corti's organ is completely terminated only after birth.

Likewise the differentiation of the tegumentum vasculosum on the entire length of the cochlear canal is terminated in the postnatal period. Its folds are made more complex and some of them branch. The internal cavity of the fold is completely filled with blood capillaries and connective tissue cells, but partially simply grows over. The tegumentum vasculosum forming the epithelial cells becomes significantly larger and the difference between the dark and light cells becomes much clearer. Even their configuration changes. The connection between epithelial cells and blood capillaries becomes more intimate. Intensity of the reaction for nucleic acid, total protein and functional groups of protein molecules increases.

The final differentiation of the cochlear canal also takes place in the postnatal period. Each ganglionic cell is surrounded by a capsule. The cellular body of a neuron significantly increases due to cytoplasm. The dimensions of a nucleus remain practically unchanged, and neurons of the cochlear ganglion are distinguished by the comparatively small nucleus. Nissl's substance appears in the cytoplasm of neurons; the clumps of it are basically located along the periphery of the cell. The intensity of reaction for RNA, total protein and functional groups of protein molecules in the cytoplasm of the neurons increases. The greatest intensity of these reactions is observed in the processes of the neuron and, correspondingly, in the nerve fibers. From the 2nd day after birth it is possible to observe differences in the intensity of reaction among 140 individual neurons in the cochlear ganglion, which usually indicates a beginning of functioning. According to morphological data the final differentiation of Corti's organ in all of its parts including the cochlear ganglion is terminated on the 15th-20th day of postnatal life.

Electron microscopic investigations of Corti's organ of a chick of 18-21 days incubation and from the 5th-9th day of postnatal life showed that differentiation of receptor and supporting cells at the base of the cochlear canal is terminated on the 15th-16th day of incubation, and furthermore a "maturation" of synapses takes place here, which is expressed by thickening of synaptic membranes, increase in the osmiophilic nature and electron density and also by the appearance of a certain number of mitochondria and synaptic vesicles in the nerve endings. Moreover a number of changes takes place in the tectorial membrane; it is thickened and fibrillar structures appear in it in place of the amorphous substance. The increase in the density of the tectorial membrane is expressed in its sharp division from the surrounding fluids by a thin plasmatic membrane. In the receptor cells themselves the cuticular lamina is strengthened, acquiring a typical definitive shape and consisting of a weakly osmiophilic homogeneous substance of average electron density. Just as in the vestibular receptor epithelium, in the receptor and supporting cells Golgi's apparatus develops very intensively, which evidently is connected with the increase in certain secretory processes. Basically the further development of Corti's organ is connected with the extension of processes of differentiation towards the summit of the cochlear canal. As was noted above, this process completely terminates toward the 15th-20th day of postnatal life (differentiation of the lagena takes place independently of the differentiation of Corti's organ). The divergence with the physiological data in regard to the end of maturation of the auditory analyzer, which relate it to the 30th day of postnatal life of the chick, in all probability is connected with the maturation of the centers and myelinization of nerve fibers.

A HISTOCHEMICAL STUDY OF SPECIFIC AND NONSPECIFIC CHOLINESTERASE

At the first stages of development (4th-7th day of incubation) the auditory vesicle gives a reaction for specific cholinesterase (acetylcholinesterase-ACE) which is somewhat stronger than in the surrounding tissues.

On the 8th day of incubation an intensive reaction for ACE on the rudiment of the ganglion appears, which increases simultaneously with growth of the ganglion, and indicates the very high activity of ACE in it. The reaction in the cochlear part of the ganglion is somewhat weaker than in the vestibular part. The reaction for non-specific cholinesterase (butyrylcholinesterase-BCE) is present but very weak.

On the 12th day of incubation a very weak positive reaction for ACE appears on the receptor formations of the inner ear; at this stage is first observed a stronger reaction with butyrylcholiniodide, which develops BCE, than with acetylthiocholiniodide, with the aid of which specific and nonspecific cholinesterase are summarily developed. The localization of enzyme on receptor structures of both

cases corresponds. The developing tegumentum vasculosum has a very weak diffuse reaction for nonspecific cholinesterase (CE). The activity of ACE is somewhat stronger in the receptor epithelium of the maculae than in the receptor epithelium of the cristae. Greater activity is manifested in the plana semilunata of the ampullae of the vertical semicircular canals than in the receptor epithelium of the cristae. The same distribution of enzymes and the same degree of intensity of reaction is preserved even on the 14th day of incubation.

Significant changes in the appearance of cholinesterase take place during the period from the 15th to the 19th day of incubation. Beginning with the 15th day, differences in the appearance of cholinesterase are increased with the aid of acetylthiocholiniodide, which summarily develops specific and nonspecific CE, and butyrylthiocholiniodide, which only develops nonspecific CE (BCE). Therefore the intensity of reaction with butyrylthiocholiniodide, naturally must always be lower than the intensity of the total reaction with acetylthiocholiniodide. However, in the embryogenesis of the structures of the inner ear of a chick embryo we first ran into a paradoxical phenomenon (which was already briefly mentioned): a reaction conducted with butyrylthiocholin for developing BCE proved to be much more intensive than the summary reaction conducted with the aid of acetylthiocholin. Only the vestibular and cochlear ganglion are an exception, for they preserve usual correlations of the phase reactions. /141

Thus with the reaction with acetylthiocholin in the region of the cochlear canal of a chick embryo of 19 days incubation the most intensive reaction is observed in the cochlear ganglion on the macula in the region of the synapsis of the nerve fibers with the receptor cells. A reaction of average intensity is observed in Corti's organ with the same localization, i.e., in the region of the synapses under the bases of the hair cells. In the tegumentum vasculosum there is a general diffuse yellowish background.

In the vestibular part of the inner ear of this stage the greatest intensity of reaction with acetylcholin is observed respectively in the vestibular ganglion. In the utricular and saccular maculae total activity of specific and nonspecific CE is also manifested in the region of synapses under the bases of the hair cells; this reaction is somewhat weaker in the peripheral portions of the utricular macula and is strengthened in its central regions. In the cristae the activity of these enzymes is also located in the region of synapses, i.e., under the bases of the hair cells. The intensity of the reaction is somewhat stronger in the cristae of both vertical semicircular canals (complex cristae) and somewhat weaker in the ampullar crista of a horizontal (lateral) semicircular canal. The region of the planum semilunatum, at the same time, gives a very weak reaction. Such a distribution of localization and degree of activity of enzymes takes place practically throughout this entire period (15-19 days of incubation) with a certain increase in the intensity

of reaction toward the 19th day of incubation.

With testing on the isolated inner ear of a chick of 15-19 days incubation for nonspecific CE with the aid of butyrylthiocholine, the weakest intensity of reaction is noted in both ganglia, which indicates that in the latter specific cholinesterase is almost exclusively localized. Nonspecific CE is present here, but to a significantly smaller degree, giving a stain which is somewhat darker than the usual background stain in reaction. An almost equal intensity of reaction is observed in both cases in the region of synapses in the lagenar macula which leads one to assume that BCE developed both with the aid of acetylthiocholine and with butyrylthiocholine is located here. However it must not be forgotten that the other case may take place here when, in the lagenar macula, both specific CE, revealed only with the aid of acetylthiocholine, and a third CE, revealed only with the aid of butyrylthiocholine, are present in equal amounts. In this case we will also have an equal intensity of reaction with both means of determination. The insignificant divergences in the localization of enzyme with both these reactions leads to the conclusion that in the given case we are dealing just with the latter assumption. The receptor epithelium of Corti's organ and the nerve fibers ascending to it give a more intensive reaction with butyrylthiocholine than with acetylthiocholine at this stage. Therefore it is possible to assume the presence of some third cholinesterase here which, in contrast to BCE, is not revealed with the aid of acetylthiocholine but only with the aid of butyrylthiocholine. It is difficult to come to a conclusion whether or not a specific CE is present here, since in the given situation it is possible to have two cases: either specific cholinesterase is absent here, which is difficult to allow, or here are contained only BCE and the third CE revealed only with butyrylthiocholine. The second of these possible assumptions seems to us to be the more probable; namely, that specific CE is present here which is revealed by acetylthiocholine in a large amount, or the more active, third CE which is revealed only by butyrylthiocholine. /142

All the parts of the vestibular apparatus, i.e., the macular and saccular maculae and the ampullar cristae of all three semicircular canals give in turn a significantly more intensive reaction with butyrylthiocholine than with acetylthiocholine. The degree of enzyme activity in them is more or less equal. This indicates the equal total enzyme activity of BCE and the third CE. The plana semilunata of the ampullae of the vertical semicircular canals are well revealed with this reaction, where evidently at this stage there is primarily the third CE.

These correlations which appear clearly on the 19th day of incubation, gradually grow from the 12th to the 19th day and are first noted in the cochlear canal. It is possible to assume that this third cholinesterase which reacts only with butyrylthiocholine and does not react with acetylthiocholine first appears on the 12th day of incubation and gradually develops to the 19th day.

In general, the described distribution of ACE, BCE and the third CE reacting with butyrylthiocholiniodide is preserved with very small changes to the 31st day after hatching, i.e., the day when, according to physiological data, the development of all parts of the inner ear of birds are almost totally completed.

In the last day of incubation at the time of pecking out and 3 days after pecking out in the vestibular and cochlear ganglion reactions only for ACE are observed. The intensity of reaction with butyrylthiocholine drops sharply and approaches the stage of the background staining of surrounding tissues. Such an arrangement is also preserved to the 31st day of postnatal life. In the postnatal period the prescribed total reaction with acetylthiocholiniodide in no way differs from that described above on the 19th day of incubation. As before, greater activity is determined in the cochlear and vestibular ganglia than in the region of synapses in the sensory epithelium of the lagenar macula in the cristae of vertical and horizontal semicircular canals and in the saccular and utricular macula, whereby in the latter enzyme activity somewhat increases on the periphery of the macula and is equally distributed in the sacculus. In the remaining parts of the inner ear uniform background staining is observed.

Upon reaction with butyrylthiocholiniodide a sharp increase in enzyme activity is observed which is evidently due to the third CE in all synaptic regions of the receptor epithelium of all receptor organs of the inner ear, without exception. It is absent only in the ganglionic cells of both ganglia, where on the contrary a sharp drop of BCE is observed. An especially intensive increase is observed in the receptor epithelium of the lagenar macula and in the /143 plana semilunata of the ampullae of the vertical semicircular canal. Enzyme activity increases very significantly upon reaction with butyrylthiocholine in the utricular and saccular macula and in the cristae of all three ampullae. Enzyme activity of BCE in the region of the planum semilunatum on one of the walls of the ampulla of the horizontal semicircular canal is diffuse in contrast to its clear localization in the planum semilunatum of the ampullae of both vertical semicircular canals. In the tegumentum vasculosum, as before, there is observed only a weak background staining, attesting to the presence of insignificant enzyme activity of BCE in it.

It is necessary to note that this third CE is not blocked by inhibitors specific for BCE; however, we were not especially occupied with the question of the nature and characteristics of the third CE. In order to determine this very precisely, quantitative and qualitative biochemical studies are necessary.

Thus investigation of cholinesterase in the process of embryogenesis of receptor structures of the inner ear of a chick embryo shows that the localization of specific cholinesterase (ACE) in receptor structures and in the ganglia of the eighth nerve changes in strict correspondence with morphological processes taking place in

them. Thus the appearance of intense activity of ACE in the rudiment of the ganglion on the 8th day of incubation corresponds to the appearance of processes of ganglionic cells which are well impregnated with silver and osmium forming nerve fibers which begin to grow up to the sensory epithelium and partially inside the latter.

Precisely the same appearance of a positive reaction for ACE on the 12th day of incubation in receptor formations of the inner ear corresponds with the beginning of the formation of synapses between nerve fibers and basal parts of receptor cells growing into receptor epithelium. The great intensity of the reaction for ACE and receptor structures of vestibular apparatus evidently is connected with the more massive synaptic apparatus of the vestibular receptor cells in comparison with the cochlear receptor cells. This assumption is confirmed by the great activity of ACE in the center of cochlear macula where there are basically Type I receptor cells with powerful scyphoid synapses.

Unfortunately it was difficult for us to draw a parallel between development of morphological structures and an increase in the activity of the third nonspecific CE which is strengthened toward the 13th day of incubation, insofar as we do not know the function of this cholinesterase, and therefore we cannot even theorize which cytoplasmatic structures it may be connected with.

HISTOCHEMICAL STUDIES OF RESPIRATORY ENZYMES

In the first half of incubation (4th through 10th day) a very strong reaction for succinate dehydrogenase (SDH) is observed in all tissues of the rudiment of the inner ear. The intensity of the reaction is very great but it takes place specifically; i.e., enzyme activity is observed only in the mitochondria. A still more intensive reaction is observed on the 10th day of incubation in the ascending nerve fibers.

Beginning with the 11th day of incubation, the decrease in intensity of reaction for SDH in all supporting and tegumental tissues of the inner ear and a concentration of the enzyme activity of the SDH in the receptor structures is observed even in the ascending nerve fibers. Also, the roof of the ampulla becomes more transparent, and weak outlines of the plana semilunata are observed even in the ampullae of the vertical semicircular canals. In all these formations activity of SDH, as before, is manifested in the mitochondria. On the 14th day of incubation enzyme activity of SDH in all /144 tissue somewhat increases, but the nerve fibers ascending to the receptor formations still stand out clearly against its background. In the cochlear canal, moreover, a very intensive reaction is observed on the "tips" of the folds of the tegumentum vasculosum. The vestibular apparatus is distinguished by its very intensive reaction for SDH of all tissues, but even so it is possible to distinguish the beginning formation of the plana semilunata in the transparent ampullae. In addition, in the utricular and saccular maculae the

strengthening of enzyme activity in their receptor epithelium, as well as in the receptor epithelium of the lagenar macula and Corti's organ, is not noted.

In the course of 15-17 days of incubation the process of "lightening" supportive structures is noted; i.e., there is a decrease in their intensity of reaction for SDH. At the same time, an increase in the intensity of reaction in the receptor epithelium of various receptor formations of the inner ear takes place. This process appears earliest in the cochlear canal, both in the lagenar macula and in Corti's organ. In the vestibular part, intensity of reaction in the peripheral parts of the utricular macula increases. As far as the ampullae of the semicircular canals are concerned, their receptor epithelium of the cristae has almost the same enzyme activity of SDH as the lining epithelium.

On the 19th day of incubation in all tissues of the inner ear of birds enzyme activity of SDH is still maintained, although even less intensive than at the preceding stages. In the cochlear canal, a high enzyme activity of SDH is noted in the cochlear ganglion and in the folds of the tegumentum vasculosum. A somewhat weaker, but still high, activity takes place in the nerve fibers ascending to the lagenar macula, and in the receptor epithelium of the lagenar macula. In the receptor epithelium of Corti's organ the reaction is significantly weaker, although it does exceed the reaction of the surrounding tissues.

In the ampullae of the vertical semicircular canal a very strong enzyme activity is observed on the periphery of the plana semilunata, evidently along the path of a blood vessel. A somewhat weaker activity is present in the ascending nerve fibers, and there is a still weaker reaction in the receptor epithelium of the cristae. In the receptor epithelium of the utricular macula in general there is observed average enzyme activity which is somewhat strengthened toward its anterior, elevated end.

In each ampulla of vertical semicircular canals the plana semilunata are connected by two narrow stria, outstanding by reason of their high enzyme activity. They pass along the base of the crista and consist of cells rich in mitochondria which surround all receptor formations of the vestibular apparatus and are especially clearly pronounced in fishes. In the receptor epithelium of the saccular macula, enzyme activity is weaker than in the utricular macula and is somewhat increased toward the periphery of the macula. An average enzyme activity is observed in the ampulla of a horizontal canal in the ascending nerve fibers and in the receptor epithelium. The planum semilunatum is not developed here. A very high activity is noted in the vestibular ganglion.

This process of the "lightening" of supportive epithelial and connective tissue structures of the membranous labyrinth, i.e., a weakening of enzyme activity of SDH in them, with a simultaneous

increase of SDH activity in the receptor epithelium continues until the moment of pecking out and in the first days of postnatal life.

Toward the 3rd day of postnatal life, epithelial and connective /145 tissue parts of the inner ear become completely transparent and colorless. Enzyme activity of SDH in the cochlear ganglion, tegumentum vasculosum, the lagenar macula and Corti's organ significantly decreases, although in the cochlear ganglion and in the nerves ascending to the lagenar macula it still remains higher than average. A significantly greater enzyme activity is observed in the vestibular part of the labyrinth and in the neurons of the vestibular ganglion. Just as in the cochlear canal, the connective tissue and epithelial parts here are transparent and completely colorless. Enzyme activity in the saccular macula increases greatly where it reaches the same degree as in the ascending nerve fibers and the blood vessels of the plana semilunata. At the same time the average enzyme activity is manifested in the cylindrical epithelium of the plana semilunata where it is also localized in the mitochondria. In the utricular macula enzyme activity of SDH is somewhat less than in the saccular macula. In both maculae enzyme activity is equally distributed along the entire receptor epithelium, being localized in the mitochondria of receptor cells, scyphoid synapses and nerve fibers growing into the receptor epithelium. In the receptor epithelium of the cristae, strong enzyme activity is observed in the ampullar crista of the horizontal semicircular canal and, as before, high activity is preserved in the ascending nerve fibers.

Upon arranging a reaction for SDH with neotetrazolium, a somewhat stronger staining of supportive tissues, giving a rose-colored background, is observed, which is connected with the dissolving of the precipitate of formyzine simultaneously forming in lipids. Upon the action with nitrocinetetrazolium, especially with conducting a piece through spirits after the reaction, such a background staining completely disappears.

Upon investigating the localization and enzyme activity of cytochromoxidase (CCO) in the embryonal development of the inner ear of a chick, what seems to be the reverse relationship of these two enzymes, which we once noted (Vinnikov and Titova, 1961) for the definitive mammalian Corti's organ, is conspicuous. While in the process of embryonal development a certain decrease in enzyme activity of SDH takes place in a number of supportive and receptor structures of the inner ear, with investigation of CCO we encounter more complex changes of enzyme activities of CCO and individual receptor formations of the inner ear.

At the early stages of development, CCO is very weakly manifested in the rudiment of the inner ear. Significant enzyme activity is manifested only on the 8th-10th day of incubation in the rudiment of the ganglion. It is difficult to guarantee the reliability of these results, since, at these stages, the entire rudiment of the inner ear is hard to isolate and, possibly, the weak intensity of reaction for

CCO is not connected with the weak enzyme activity but with the poor penetrability of tissues for the incubation medium. In general, revealing CCO in embryonal tissues is practically possible only with the application of the Japanese modification of Nadi's reaction.

On the 16th day of incubation, when the inner ear can already be isolated from the cartilaginous capsule surrounding it, a high enzyme activity of CCO on receptor structures is manifested in it. In it the greatest CCO activity is observed in neurons of the cochlear and vestibular ganglia and in the nerve fibers ascending to the receptor epithelium. In the cochlear canal a greater enzyme activity of CCO is observed in the folds of the tegumentum vasculosum than in the receptor epithelium of Corti's organ and a weaker reaction in the lagenar macula. In addition, the epithelial and connective tissue part of both the cochlear canal and the vestibular region of the labyrinth remain completely transparent and colorless. In the vestibular part of the labyrinth a significantly stronger enzyme activity in the receptor epithelium of both maculae in comparison with the receptor epithelium of Corti's organ and the lagenar macula is observed. However, while an increase in enzyme activity of SDH was observed on the periphery of the maculae, the enzyme activity of CCO in the utricular and saccular macula has an average value on the periphery and a very great value in the center of the maculae. In the crista of a horizontal semicircular canal, which has the form of a tapered tongue of flame, a rather high enzyme activity of CCO over the entire receptor epithelium, uniformly covering the entire crista, is noted. In the ampullae of the anterior and posterior vertical semicircular canal, in which complex cristae are located, the distribution of CCO is more complex. Enzyme activity of CCO is manifested in small portions along the upper crest of the cristae and on three projections of the septum cruciatum. But the enzyme activity of CCO on these small portions is very strong. On the plana semilunata of these ampullae, in their epithelium, enzyme activity of CCO is weak, and it appears to be eroded and indistinctly outlined. At the same time vessels of the region of the plana semilunata are not developed. /146

On the 18th day of incubation, enzyme activity of CCO is increased on the receptor epithelium of Corti's organ and the lagenar macula; i.e., in the receptor formations of the cochlear canal. In the utricular and saccular maculae and the ampullar cristae, i.e., all the receptor formations of the vestibular part of the labyrinth, enzyme activity of CCO falls. On the 19th day of incubation the drop in enzyme activity encompasses even the tegmentum vasculosum, but it is somewhat increased in the lagenar maculae. In the ampullae of the vertical semicircular canals, the intensity of reaction in the plana semilunata is increased.

Toward the end of the embryonal development, on the 21st day of incubation, an increase of enzyme activity of CCO in all receptor structures of the cochlear canal is observed. In the vestibular apparatus enzyme activity in the receptor epithelium of the

crista is increased, as well as that in the lining epithelium and in connective tissue portions of the inner ear.

A still greater increase in enzyme activity in all receptor structures of the inner ear, tegumentum vasculosum and epithelium of the plana semilunata is noted at the time of pecking out. An especially strong elevation of it is observed in the lagenar macula, and a somewhat smaller rise in the receptor epithelium of Corti's organ. However, at the same time a drop in enzyme activity is observed in the receptor epithelium of the utricular macula, which may be attributed to the increased function of this organ.

In neurons of the cochlear and vestibular ganglion a high enzyme activity of CCO is maintained.

On sections through the cochlear canal it is seen that reaction toward development of enzyme activity of CCO takes place exclusively in the mitochondria of receptor cells and nerve fibers. At the same time, mitochondria of supporting cells do not appear. Sensory hairs of receptor cells remain colorless. Mitochondria swell somewhat with this reaction, although less than with the classical Muh reaction.

On a section through the lagenar macula the pattern of CCO distribution is the same. Only in receptor cells of the lagenar macula mitochondria appear to be stained more intensively. In nerve fibers ascending to them, round mitochondria are distributed more densely and are stained more intensively. The tectorial membrane of Corti's organ remains completely colorless at all stages, as does the basilar membrane.

/147

In the receptor epithelium of the utricular macula CCO is located just as it is in the lagenar macula, but the reaction in the former is less intensive.

In neurons of the cochlear and vestibular ganglia all the cytoplasm is filled with round, darkly stained mitochondria, as are the processes proceeding from them.

Thus, during the development of the inner ear of birds a strengthening of the enzyme activity in neurons and nerve fibers is observed.

DEVELOPMENT OF THE INNER EAR OF MAMMALS

Development of the Vestibular Apparatus

The inner ear of mammals, just as the inner ear of birds, is separated into vestibular and auditory parts, which are distinguished not only by the anatomical structure of their receptor formations but also by a fine structural difference between the receptor cells lying at their bases. As was already noted in the literary survey,

at the base of sensory structures of the vestibular apparatus lie Type I and Type II receptor cells, while in Corti's organ the basic receptor elements are external and internal hair cells.

An investigation of the development of the inner ear of mammals was conducted on embryos of goats, Astrakhan sheep, rabbits and cats. A study of the development of the inner ear of goat and sheep embryos, especially in the early stages, has the advantage that in these animals the developmental process is more extended in time than in the majority of laboratory animals (intrauterine development of sheep and goats is around 150 days, but that of the rabbit is 30 days). However, the difficulty of obtaining material, especially at the later stages of development, and its use for histochemical reactions compelled us to switch to prematurely born laboratory mammals (rabbits and cats) for histochemical studies, for in these animals the final stages of differentiation of the inner ear take place primarily in the postnatal period.

In the goat and Astrakhan sheep (9 - 10 mm in parietococcygeal length) already all parts of the vestibular apparatus are established. The rudiment of the receptor epithelium of the utricular sacculus is arranged in the form of two clearly defined parts; the separation of rudiments of receptor epithelium of the ampullar cristae takes place somewhat later. The surface of the remnant of the receptor epithelium of the maculae is uneven, but the sensory hairs upon its surface could not be detected under a light microscope. In the central portions of these rudiments above the epithelium lies a narrow homogeneous stria, giving an intense reaction for total protein and functional groups of protein molecules. In all probability this is the rudiment of the covering formation. Sometimes certain outgrowths proceed from this stria. There are backings of the semicircular canals and ampullar expansions at their ends. In the rudiment of the receptor epithelium, on preparations mixed with osmium and stained with ferric hemotoxalin, light and dark narrow, spindle-shaped cells are seen stretched through the entire thickness of the epithelial layer.

On silvered preparations from embryos of Astrakhan sheep and goats 12 - 13 mm in parietococcygeal length, nerve fibers which are well impregnated proceed from the vestibular ganglion in the direction toward the brain. A small number of impregnating nerve fibers proceeds from the ganglion toward the receptor epithelium. The vestibular ganglion appears to be significantly more mature than the cochlear ganglion, in which no nerve fibers impregnated with silver are yet seen. Neurons of the vestibular ganglion are small, but neurofibrils which are impregnated with silver are already visible in their cytoplasm. /148

In the sensory epithelium of the rudiment of the utricular macula a subdivision into receptor and supporting cells is observed. All the receptor cells have a cylindrical shape and are located in the upper third of the epithelial layer. On preparations impregnated

with silver it is apparent that the peripheral processes of the neurons not only enter inside the receptor epithelium, but even approach the base of the receptor cells.

In embryos of Astrakhan sheep and goats 13 - 14 mm long it is possible to see how the nerve fibers entwine the bases of the hair cell. But at this stage there is still no formation of scyphoid synapses. At the same time, above the receptor epithelium of the utricular macula the formation of the gelatinous part of the otolith begins.

In 15 - 16 mm embryos of the same animals the plexus (plexus externus) begins to be formed; it is located between the bases of receptor cells and supporting cells. This plexus is formed from very thin nerve fibers whose number and thickness of distribution gradually increase. The formation of the plexus begins in the middle portions of the utricular macula and gradually, according to the degree of their differentiation, spreads toward the peripheral sections. The development of the saccular macula passes through the same stages as the utricular macula but is somewhat retarded. Development of the saccular macula and ampullar cristae of the semicircular canal in Astrakhan sheep embryos 15 mm in length corresponds in stage to the development of the utricular macula of embryos of the same animals 12 - 13 mm in length.

The same structural patterns of the utricular macula are preserved even in an embryo 40 mm in parietococcygeal length, but they already encompass all parts of the vestibular apparatus. In the maculae the region which is occupied by receptor cells and the nerve plexus located under them is enlarged. The receptor epithelium of the cristae after this stage is characterized by subdivision into receptor and supporting cells. The apical parts of receptor cells are distinguished by the strong vacuolization, evidently related to processes of cupula formation.

In the most advanced embryos of Astrakhan sheep and goats (105 mm in parietococcygeal length) investigated by us, in the sensory epithelium of the utricular macula we noted very distinct differences between receptor and supporting cells. As was already noted, receptor cells are located in one distinct row in the upper third of the sensory epithelium. Cellular bodies of cylindrical shape and large round light nuclei are seen in them. The supporting cells stretch from the basilar membrane to the apical surface of the receptor epithelium. The upper, extremely contracted cytoplasmic parts of supporting cells are found between the bodies of the receptor cells. The part of the supporting cells containing nuclei occupies the lower half or two-thirds of the epithelial layer. Their small dark oval nuclei are very densely arranged in 2-3 rows. The cellular margins of supporting cells cannot be traced. In the central portions of the utricular macula sensory hairs are visible on certain receptor cells. Above them lies a compact mass of the developing otolith membrane, but otoliths (otoconia) are still not apparent.

Approximately the same structure characterizes the receptor epithelium of the ampullar cristae of the vertical semicircular canals. The basic difference lies in the intense vacuolization of the apical parts of the cytoplasm of receptor and, possibly, supporting cells, and in the absence of clearly defined sensory hairs. Above the surface of the sensory epithelium there is an already significantly developed cupula, which has a clearly defined (on fixed preparations) subcupular space. The cupula has a very even base, bounded by the subcupular space, and it seems to consist of traces intertwining in the form of a reticulum. /149

We succeeded in performing only an insignificant number of cytochemical reactions for nucleic acid and total protein on this material. As a general conclusion we may note that the early stages of development of the vestibular apparatus (goat embryo 11-12 mm in length) are distinguished by the significantly higher concentration of total protein and nucleic acid than the later stages. According to the amount of differentiation, the process of nerve fiber ingrowth connected with it and their contact with the bases of receptor cells, a certain decrease in the concentration of these substances is observed, although their content generally remains rather high. While in the receptor cells the concentration basically drops, in supporting cells the RNA concentration primarily decreases while the chromatin of the nuclei is distinguished by a rather high DNA content. The most intense drop in intensity of reaction for RNA is observed at the point of the neuroplexus (plexus externus) under the bases of receptor cells which is practically colorless; i.e., free from RNA. The intensity of reaction for total protein decreases more or less uniformly along the entire thickness of the epithelial layer.

In a rabbit fetus at 17 days of intrauterine life, all the parts of the vestibular apparatus are already present, but the process of histological differentiation has just begun in them. The receptor epithelium of the utricular macula is the most highly developed. In the center of this macula it is possible to observe a subdivision of the cells comprising it into receptor and supporting elements, while on the periphery of the macula this process has only just begun. Receptor cells are located along the apical margin of the macula, and their bases do not reach the basilar membrane, occupying approximately the upper third of the thickness of the epithelial layer. All receptor cells have a cylindrical shape. Their nuclei are light and round in shape. The body of supporting cells passes through the epithelial layer from its external surface to the basilar membrane; these cells have dark oval nuclei located in the lower portion of the epithelial layer on the bases of the receptor cells. On the apical end of both receptor and supporting cells there is a darker thickening; possibly this is the developing cuticle. The summits of sensory cells bear bundles of "claw-like" bent hairs of unequal length on their surfaces, over which lies the developing otolith membrane, represented on fixed preparations by a detritus-like substance and a few small otoliths (otoconia).

The subdivision of cellular elements of the receptor epithelium into receptor and supporting cells is especially clear upon reaction for nucleic acids due to differences in the concentration and correlation of DNA and RNA in them. While in the nuclei of receptor cells there is observed a rather low concentration of DNA and a significantly higher concentration of RNA in their cytoplasm, in the supporting cells the reverse relationship is observed: a very high concentration of DNA in their nuclei and a low concentration of RNA in their cytoplasm. The cuticle, hairs and otolith membrane give a negative reaction for four nucleic acids.

The concentration of total protein is more or less homogeneous /150 along the entire thickness of the receptor layer. It is approximately equal even in the chromatin of the nuclei and in the cytoplasm of receptor and supporting cells; the reaction in the sensory hairs has the same intensity as in the cytoplasm. The most intense reaction is observed in the cuticle. However, with reaction for functional groups of carboxyl groups of protein molecules the receptor cells stand out sharply against the background of all other elements, due to their high concentration in the cytoplasm, nucleus and cuticle. On the other hand, the content of thiol groups is insignificant in all parts of the receptor epithelium. It is somewhat increased in the cuticle and in the sensory hairs and is highest in the nerve fibers ascending to the receptor epithelium.

The vestibular ganglion at this stage is already quite well developed. Its large neurons have a large oval-round vesiculiform nucleus with a well defined nucleolus. Well developed nerve fibers and cell satellites are clearly seen. In the nerve bundle, nuclei of Schwann's cells are quite distinct. The bodies of the neurons are rich in RNA, total protein and especially in carboxyl groups of protein molecules. The concentration of DNA in the nuclei is small, but the nucleolus stands out very clearly, especially with protein reactions in which the central portion of the nucleolus is usually distinguished from its perinucleolar part.

The subdivision of cellular elements of the utricular macula into receptor and supporting elements generally terminates on the 25th day of intrauterine life. Toward the 28th day the rising nerve fibers form synapses on the receptor cells. The formation of scyphoid synapses and pitcher-shaped Type I cells (Fig. 67) is observed. In the otolith membrane toward this time there appears a large number of otoliths which are differentiated into large, medium and small sizes. All otoliths give a strong nonspecific staining by pyronin. The earlier-described cytochemical characteristics of individual elements of the utricular macula are preserved in general.

A retardation in differentiation of the receptor epithelium of the crista is observed. The morphological and cytochemical characteristics of their receptor epithelium on the 25th day of intrauterine life approximate the stage which was described for the utricular macula of a fetus at 17 days of intrauterine life. In this same

stage the separation of high cylindrical epithelium of the plana semilunata takes place in the ampulla of the semicircular canals. Toward the end of intrauterine development there are well defined sensory hairs on all receptor cells of the cristae, but no cuticle is apparent on the surface of receptor cells.

The first day after birth appeared to be a significant stage in the cytological differentiation of the utricular macula. The presence of pitcher-shaped receptor cells with scyphoid synapses (Type I receptor cells) in the utricular macula is most characteristic of this stage. Upon reaction for nucleic acid the cytoplasm of receptor is vacuolized and grainy. The concentration of RNA in them is very great, especially in the pitcher-shaped cells. The sensory hairs give a sharp nonspecific staining by pyronin. The irregularly round nuclei of receptor cells are specifically stained by pyronin and galocyanin. The nucleoli are very clearly defined and rich in both DNA and RNA. The otolith membrane, otolith hairs and cuticle lack nucleic acids. Upon reaction for total protein and carboxyl groups of protein molecules, pitcher-shaped cells, whose nuclei and cytoplasm give very intensive reactions, are especially outstanding. Moreover, their scyphoid nerve endings remain practically colorless or give a reaction of exceptionally weak intensity. A high intensity of reaction is observed in the cuticle and, on the other hand, a very weak reaction is given by the cytoplasm of supporting cells, otolith membrane and otolith. Thiol groups of protein molecules give a more intensive reaction in the pitcher-shaped receptor cells in the cuticle and especially in the sensory hairs and in the nerve fibers. /151

Along the periphery of the utricular macula there is one layer of cylindrical cells with elongated oval nuclei, which according to its histochemical characteristics is similar to supporting cells of the receptor layer. From the surface of these cells proceed non-branching fibers entering the otolith membrane. Evidently these fibers serve for its attachment.

The receptor epithelium of the crista is distinguished by the fact that pitcher-shaped cells are still insufficiently clearly defined in it. All receptor cells of the crista give a very intensive reaction for protein and functional groups of protein molecules, whereupon the nucleus and the cytoplasm of the cells are almost indistinguishable from one another by their intensity of reaction. In contrast to the earlier-described patterns, a very intensive reaction for protein is observed not only in the chromatin of the nucleus but also in its karyoplasm. The karyoplasm of supporting cells does not give a reaction for protein, and the cytoplasm of these cells shows a significantly lower intensity of reaction for total protein and functional groups of protein molecules. Nerve fibers ascending to the receptor layer are significantly darker than the Schwann's glia accompanying them. The basal membrane is easily traced only in the lateral portions. The epithelium covering the lateral portion of the crista is represented by narrow cylindrical

cells with round-oval "turbid" nuclei having transparent light granulated cytoplasm. The distribution of nucleic acids in the receptor epithelium of the crista is close to that which was described for the utricular macula.

The vestibular ganglion at this stage is close to the definitive structure. Ganglionic cells are surrounded by well defined capsules and cell-satellites. The most characteristic change in the morphology and histochemistry of neurons is the appearance of clumps of tigroids, which are still represented in a small number and are located along the periphery of the neurons. Reactions for nucleic acids, total protein and functional groups of protein molecules in the cytoplasm of neurons are significantly more intensive than in the receptor epithelium. However, individual neurons of these reactions have different intensities. Against this background the darker clumps of tigroids always stand out clearly. All these reactions are very intensive in the nucleolus, especially in the central portion (Fig. 68).

Three days after birth the receptor epithelium of the utricular macula differs little from that of adult animals. Practically no morphological changes can be observed in it. As far as its histochemical characteristics are concerned, the distribution of nucleic acids, total protein and functional groups of protein molecules changes somewhat. Upon reaction for carboxyl groups the most intensive staining is observed not only in the nucleolus but also at the apical portion (i.e. the neck) of the pitcher-shaped receptor cell, in the cuticle and in the nuclear membrane. Intensity of reaction for total protein on the part of the entire receptor layer increases strongly; it is especially high in the sensory hairs. An alternation of dark and light nuclei is formed in the layer of receptor cells, which belong, respectively, to the pitcher-shaped and cylindrical cells.

The structure of the receptor epithelium of the cristae toward the third day of postnatal life is the same as that of receptor epithelium of the utricular macula.

In neurons of the vestibular ganglion a strong elevation of intensity of reaction for total protein, especially for RNA, is observed. According to the intensity of reaction for total protein and for nucleic acids, these neurons differ sharply from the paler satellites and Schwann's syncytium. As before, the nucleoli remain very bright and clear. /152

By comparing the morphological data which we obtained from observing the development of the vestibular apparatus of the rabbit with the physiological data obtained by G.A. Obratzsova (1961) on the development of the vestibular function in ontogenesis of this animal, we may conclude that Obratzsova's conclusion that in the rabbit the ontogenesis reflexes, whose source is the phylogenetically older otolith apparatus, develop earliest of all, and only somewhat

later do reflexes arise as a result of stimulation of the phylogenetically younger semicircular canal apparatus, finds confirmation in the nonsimultaneous cytological differentiation of these receptor formations. But there is no doubt that these physiological data depend also on the development of centers and conducting paths as well as on the degree of differentiation of peripheral parts of the static analyzer.

Development of Corti's Organ

In sheep and goat embryos 9 mm in parietococcygeal length and in a rabbit embryo at 17 days of intrauterine life, the cochlear canal has the form of a short, blindly closed tubule having the appearance of a narrow oval in cross section. Its base is constructed of multinucleated epithelium and the roof of 1-2 rows of cubic epithelium. No signs of cellular differentiation are apparent at this stage (Fig. 69). The same structure of the cochlear canal is preserved even in goat and Astrakhan sheep embryos 11 mm in length, in which the cochlear canal begins to bend. It is only possible to note that the nuclei of the cells of the multinucleated epithelium begin to be crowded toward the base and that their supernuclear cytoplasmic part increases in size. The cytoplasm is intensely vacuolized. On the surface of these cells there is a darker cuticle. The epithelial cells at this stage are very rich in RNA, DNA and proteins. The mesenchyma lying around the cochlear canal becomes very dense, but the process of cartilage formation has still not begun. On preparations impregnated with silver it is seen that the greater portion of the cochlear ganglion is not yet differentiated, and nerve fibers still cannot be traced to it. However, in its dorso-caudal portions there are already differentiated neurons and nerve fibers which may be stained with silver, which ascend to the multinucleated epithelium of the base and begin to grow into it.

In a 40 mm embryo the cochlear canal already makes 1.5 helices, and in axial section of the cochlea 3 cross sections of the cochlear canal at various levels (helices) are seen, which are found in various stages of differentiation. Numerous mitoses are seen in the epithelium. In axial section through the posterior end of the basal helix it is seen that the multinucleated epithelium of the base forms two tubers with a small fissure between them at the beginning of the formation of the tunnel. Under it in the connective tissue is found the large arterial vessel (larger in the cat and rabbit than in the goat and Astrakhan sheep). In epithelial cells of the axial tuber the nuclei are distributed very densely but in a somewhat disorderly way. Their karyoplasm is intensely basophilic. The axial ends of the cells are expanded flabellately. Their surface is uneven. A dark homogeneous film is seen above them which is evidently Nissl's stage of formation of the tectorial membrane. Two cells with large ellipsoidal nuclei adjoining one another are seen in the fissure located between the tubercles. A small vacuole is seen in the cytoplasm beneath them. Below it lie two small, round basally-distributed nuclei. The cytoplasm of all these cells is light and is hard

to trace. Evidently this is the initial stage of formation of the tunnel and cell columns. In the lateral tubercle located behind the fissure three large cells with large oval nuclei and well defined nucleoli are located apically; these are evidently the future external hair cells. But there are still no hairs upon them. Under them and clearly separated from them lie three cells with large centrally located nucleoli, evidently the future Deiter's cells. Under these cells lies a distinct layer of small very dark nuclei surrounded by a very thin layer of cytoplasm which is not distinguished structurally from the nuclei of the connective tissue cells. However, these cells belong to the epithelium and under them lies a well defined basal membrane separating the epithelium from the connective tissue. The underlying connective tissue is significantly concentrated and lies in 1-2 layers. The future Hensen's cells and Claudius' cells are still not differentiated and are represented by a double-layered epithelium constantly turning into a single-layered cubic epithelium with large light nuclei. The cubic epithelium of the future vascular stria and, in part, of Reissner's membrane is present consisting of light and dark cells, thus related to an equal degree to both the cytoplasm and to the nucleus.

In an embryo of 66 mm in parietococcygeal length (Astrakhan sheep, goat) the cochlear canal makes 2.5 turns, and in axial section of the cochlea already 5 cross sections of the cochlear canal, found in various stages of differentiation, are visible. Around the cochlear canal the formation of the capsule from the hyaline cartilage begins.

The formation of external cell columns begins in the fissure described in the preceding stage. They are distinguished by thin, characteristically bent bodies and a basally located nucleus. In addition, there is a drop in the concentration of RNA, total protein and its functional groups. The concentration of DNA is preserved for a while at a rather high level. The cells of the internal columns are differentiated somewhat later. At the same time there occurs a separation of internal hair cells, which here have the shape of simple cylinders with round bases.

At the apical end is the developing cuticle, rich in proteins and their functional groups. Sensory hairs develop in the center; they are glued together in the shape of a cone. Both the cuticles and the sensory hairs are poor in RNA. The three rows of external hair cells lying in a lateral direction from the external cell columns are gradually differentiated in the direction from the third row to the first. The formation of cuticle in sensory hairs which are rich in total protein and SH + SS-groups and poor in COOH-groups takes place sequentially. RNA is practically absent in them. Their large round nuclei are shifted toward the basal round portions of the cell. The supernuclear part gradually narrows and the cell acquires the typical pitcher shape. RNA is moderately concentrated in both the cytoplasm and the karyoplasm, but both the cytoplasm and the nucleus are very rich in COOH-groups of protein. Between the

bases of external hair cells and the summits of the underlying Deiter's cells a space forms which Engström (1960a) associates with the formation of a special Corti's lymph. In the supporting Deiter's cells the nuclei are round and smaller. The nuclear membrane is rough, and the large chromatin grains are rich in DNA. The karyoplasm is stained with galocyanin, but this staining is removed by treating the tissue with ribonuclease. /154

The spiral limb consists of dense connective tissue with well defined cellular elements. The region of the future spiral incisure is represented by multinucleated high cylindrical epithelium. In goat and Astrakhan sheep embryos, vacuolization is observed in its center at this stage, i.e., beginning of the process of epithelium resorption and formation of the spiral incisure. In the rabbit and cat these processes begin at a later stage of development of Corti's organ and take place in the postnatal period of ontogenesis. The future vascular stria is represented by a still undifferentiated epithelium, sharply separated from the underlying connective tissue by a basal membrane. Above the future Reissner's membrane processes of resorption of connective tissue begin, leading to the formation of perilymphatic space. A vessel grows in at the point of the future prominentia spiralis. The epithelium in this place thins out somewhat, but the characteristic curvature is still lacking.

Neurons of the spiral ganglion at this stage have rather large dimensions. The large round nucleus is located eccentrically. There is little DNA in it. The nuclear membrane and the cytoplasm are rich in RNA, protein and COOH-groups. The nucleolus is especially rich in COOH-groups; its rim is violet and the internal contents are reddish.

In newborn kitten and rabbit it is possible to see various stages of differentiation of Corti's organ on different helices of the cochlea. However, the hair cells themselves are already almost completely differentiated, especially on the lower helices. The development of the auxiliary structures of Corti's organ occurs chiefly in the postnatal period.

On the basal helix of the cochlea of a newborn animal the spiral lip is constructed of dense connective tissue and is covered from above by a very loose epithelial layer of light vacuolized cells, the broad base of which lies on the basal membrane. The nuclei are round-oval and light, with 1-3 and even more nucleoli rich in RNA, DNA, protein and COOH-groups. Their bases are in close contact with one another but the summits diverge flabellately and are in contact in the region of the cuticle, where fibers of the tectorial membrane proceed from them. This gives them the characteristic "lacey" appearance. At a certain distance from the point of origin of the tectorial membrane, large irregularly shaped nuclei of the epithelial cells are very highly elevated and are rich in DNA and COOH-groups. The cytoplasm of the cell is very transparent and contains COOH-groups in a diffuse state. They are located in 1-2 rows.

These patterns automatically lead to a consideration of the total conversion of epithelial cells into elements of the tectorial membrane. The latter is constructed of diversely arranged fibrils, undoubtedly of protein nature, which are distinguished by their wealth of total protein and its functional groups. The fibrils are sealed in some kind of cement, giving a bright positive Schick reaction and relating evidently to polysaccharides. Protein, its functional groups and RNA are practically absent. There is not even any RNA in the fibers of the tectorial membrane. The tectorial membrane at this stage reaches the tunnel.

In the spiral lip at the point of the future spiral incisure is located a very even multinucleated layer of high cylindrical epithelium separated from the spiral lip by a well defined basal membrane. Nuclei of these epithelial cells is round-oval and transparent with an eccentrically located nucleolus. Cellular margins are weakly distinguished. Inside the cells there are fibers with a high content of COOH-groups.

The internal hair cells located along the sides of the cells have an irregular pyriform shape. Their broad base lies on the bodies of the internal supporting cells. The cytoplasm of internal hair cells is transparent, weakly granulated and relatively poor in nucleic acids and proteins. COOH- and SH-groups are contained in the granules, fewer in the internal hair cells than in the external ones (Fig. 70). There are especially few of them in the nucleus and in the subnuclear zone.

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The portion corresponding to the cell columns is located lateral of the internal hair cells. There is still practically no tunnel at this stage, and in its place is seen a fibrous vacuolized cytoplasm. However the cell columns are already separated at their bases; at the same time the internal cell columns preserve their vertical position and the external ones form an angle. The coracoid and oar-shaped processes have already begun to be differentiated. In the cytoplasm of cell columns there are longitudinal fibrils rich in protein and its functional groups. The round large nuclei of cell columns are located at the very base. They contain 1-2 nucleoli and are poor in DNA and protein.

The external hair cells lying in 3 rows form a distinct angle in relation to the tunnel. They have a pyriform shape and are located rather close to one another, although they are separated by phalanges. The nuclei are large and oval-round, quite rich in DNA, protein and COOH-groups. On the surface of the cells a cuticle develops with sensory hairs which are poor in COOH-groups but contain a great deal of SS- and SH-groups.

The round bases of external hair cells lie on the scyphoid summit of Deiter's cells. The latter, just as the external hair cells, are located in 3 rows, but their broad thickened bases lie on the future basilar membrane. Their phalangial processes bend around the

bodies of the external hair cells and proceed to their summits, where they terminate in the backing of the phalanges.

The Hensen's and Claudius' cells located behind the external hair cells are still completely undifferentiated and are arranged in several rows. In their large oval nuclei the nucleoli have a marginal location. There is little cytoplasm and they are sharply vacuolized. In the apical part there are granules with a high concentration of COOH-groups and RNA and a relatively small concentration of protein. Claudius' cells form an angle with the external spiral incisure and are represented by one layer of cylindrical elements with round transparent nuclei located at the base of the cell. Their cytoplasm is vacuolized and rich in RNA. A well defined basal membrane separated Claudius' cells from the connective tissue. At the transition point of Claudius' cells into the epithelium of the vascular stria the protrusion of the prominentia spiralis is formed, in which the indications of secretion are clearly visible.

The epithelium of the vascular stria is characterized by the alternation of dark and light cells. The cytoplasm in both of these is vacuolized. This difference appears sharply with the development of RNA, total protein and COOH-groups but is completely removed with the aid of SS- and SH-groups. Vessels of the vascular stria are located in the connective tissue and turn into the epithelial layer. The typical basal membrane is absent here. Connective tissue is represented by loose layers of their fibroblasts and of a fundamental substance and are abundantly provided with blood vessels.

Reissner's membrane separating the perilymphatic space from the endolymphatic space consists of two layers: an epithelial one, turned towards the cochlear canal, and a connective tissue one, turned toward the vestibular scala. Epithelial cells are thickened; their /156 cytoplasm is vacuolized and the vacuoli are distributed in the form of brushes. The nuclei are round-oval with finely divided chromatin. The margins of the cell are clearly visible. Connective tissue cells are poor in cytoplasm. The protein and COOH-groups are contained chiefly in the nucleus and the nucleolus.

Claudius' cells, Hensen's cells and the rest of the remaining portion of the developing Corti's organ, and also the spiral incisure, are located on the future basilar membrane forming characteristic S-shaped curves, at the summit of which is located Corti's organ. The basilar membrane at this stage consists of connective tissue elements which are distributed in 3-4 rows. There are still no fibrillar structures in it. There is no endothelium from the side of the scala tympani. Connective tissue cells are unusually rich in nucleic acids, proteins and their functional groups. The basal membrane between the epithelial cells of Corti's organ and connective tissue elements of the basilar membrane is well developed and has a clearly defined fibrous structure; in all probability it is the backing of the fibers of the future basilar membrane.

During the first week of postnatal life, development of the tectorial membrane is continuous. At the same time, a large portion of the epithelium of the spiral incisure is resorbed (Fig. 71a). Processes of resorption begin from the central portions of the rudiment. In kittens and rabbits during the second week of postnatal life the spiral incisure which has formed is lined with a flat endo-
thelium, the cells of which are poor in protein, its functional groups and nucleic acids (Fig. 71b). Due to the formation of the spiral incisure a tightening of the basilar membrane takes place, and intensified processes of differentiation begin for both its fibrillar and its connective tissue structures. The tectorial membrane is already completely differentiated at this time. It forms a free cupula over the spiral incisure and overlaps both the internal and external hair cells proceeding to Hensen's cells (Fig. 72). Its fibrils have a different direction in the central and peripheral portions and different concentrations of protein and its functional groups which decrease in the peripheral zone. Fibers of the tectorial membrane are especially rich in SS- and SH-groups.

Reissner's membrane is completely differentiated on the basal helices at this time. The RNA content in the cells of Corti's organ itself and in its auxiliary structures become more uniform, with the exception of Claudius' cells and the epithelium of the vascula stria which are unusually rich in RNA.

At the latest stages, with the transition to the definitive state, RNA in the external hair cells is organized around the nucleus. Differentiation of Corti's organ and its auxiliary structures on all helices of the cochlea terminates on the 10th-12th day of postnatal life. The spiral ganglion of all helices is also completely differentiated at this time (Fig. 73). The large ganglionic cells contain large, round, well-structured nuclei with large round nucleoli. Their cytoplasm contains agranulated Nissl's substance rich in RNA and proteins which is variously distributed in different ganglionic cells. Ganglionic cells are also heterogeneous in their content of protein and in functional groups. The given short description of histogenesis of Corti's organ shows that a definite step-by-step process is observed in its development. Differentiation of the perceiving hair cells and those auxiliary elements which have a supportive and secretory function takes place in the first step. The beginning of differentiation of sensory cells is connected with the growth of nerve fibers from the adjacent portion of the spiral ganglion up to them. This allows the possibility for a newborn animal to perceive auditory stimulation of general nature. The frequency perception of sounds characteristic for the given type of animal is not only connected with the preparedness of the hair cells for perceiving adequate stimulation, but also with resonating motions of the basilar membrane which are made possible toward the end of the second week of postnatal life; i.e., at the time when not only the hair cells but also the auxiliary structures of Corti's organ are completely developed.

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Changes in nucleic acids, the so-called general or total protein and its functional groups, also possess several peculiarities. Before the beginning of the processes of cytological differentiation cells of the backing of the organ are unusually rich in proteins and nucleic acids. Toward the end of the differentiation processes these reserves are depleted, but as a rule the concentration of these substances in the cell is somewhat elevated before the beginning of the receptor or secretory function; only at this time does redistribution of these substances within the cell take place, i.e., that chemical structural relationship which is characteristic for the given type of cell is formed. A study of the embryonal development of the inner ear of mammals is of great interest. Just as in birds, in mammals there is a special section of the inner ear perceiving vibrations of the aerial medium in the band of auditory frequencies, a so-called Corti's organ, but it differs from the organ of hearing in birds by a large number of substantial characteristics, both in fine microscopic and submicroscopic structure and in the chemism and macroscopic structure. These differences in general are so important that they allow us to assume that their perception of auditory frequencies is possibly based on somewhat different principles. During this study we have investigated the development of the inner ear of mammals, including Corti's organ, by methods of general histology, neurohistology and cytochemistry. However, electron microscope investigations of the development of Corti's organ in mammals and embryogenesis and in postnatal period are very interesting, but methodologically very difficult work; it may be completed in the future as an independent investigation.

In the process of ontogenesis of the inner ear in mammals it is possible to note the following peculiarity: while individual anatomical parts of the inner ear are isolated almost simultaneously (since the subdivision of the auditory vesicle into two sacs can hardly be considered to be exclusively the backing of the otolith apparatus), their cytoplasmatic differentiation occurs nonsimultaneously; first the maculae begin to be differentiated, then the ampullar cristae and the auditory parts of the inner ear.

In parallel with the morphological differentiation, a drop in the concentrations of RNA, total protein and its functional groups is observed which, before the beginning of a functioning, is replaced by a process of accumulations of these substances. Moreover, differentiation of the cells is accompanied by a redistribution of the biologically active chemical substances, which is especially noticeable with the formation of the cuticle and sensory hairs, with the process of nerve fiber ingrowth, with the formation of tigroids in the maturing neurons, etc.

It is rather difficult to compare in stages the development of the vestibular part of the inner ear with the auditory part in mammals. In general, the vestibular part of the labyrinth develops and is differentiated as a whole, and those divergences which are observed in the cytological differentiation of its individual parts in

the end come down to a single process of differentiation of the organ, which is terminated toward the third day of postnatal life of the rabbit. The auditory part of the labyrinth is differentiated in the process of development by the fact that in it processes of differentiation of the individual portions are separated not only in time but in space also. While in the lower portions of the cochlear canal differentiation of Corti's organ is practically completed, the summit of the cochlea is found in the later stages of differentiation. Thus the preparedness of Corti's organ for perceiving sounds of different frequencies hardly begins simultaneously. Morphological differences in the development of the vestibular and auditory parts of the inner ear may be basically reduced to the following. In the vestibular organ the development of the receptor part and auxiliary structures takes place simultaneously. Therefore, when in the newborn rabbit final differentiation of both types of receptor cells (both cylindrical and pitcher-shaped) takes place, the organ is completely ready to function. In the auditory part of the inner ear, on the other hand, processes of differentiation of receptor structures are almost completely terminated at the moment of birth, but processes of differentiation of auxiliary structures of Corti's organ are terminated only on the 10th day of postnatal life, and only after this is the organ ready for the perception of sounds of differing frequency. /158

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The development scheme of the membranous labyrinth (inner ear) is similar in its general features in all vertebrates. The first paired labyrinth rudiment appears in the form of a thickening of endoderm lying along both sides of the still open neurolamina, in the region of the future hind brain, the so-called auditory placodes. Due to the descent of the central portion into the underlying mesenchyma, the placode is gradually converted first into the auditory fascia and then into the auditory vesicle. During a certain length of time the fascia remains connected with the external ectoderm and the external medium. Then this opening closes and the fascia is separated from the ectoderm. On the opposite medial wall of the auditory vesicle a protrusion is formed, i.e., the rudiment of the endolymphatic diverticulum. The auditory vesicle is lengthened in the dorso-ventral direction due to the multiplication of cells of the auditory vesicle wall and their dilation to increase in the volume of liquid filling the vesicle. The medial wall of this vesicle is thickened, becomes multi-layered and is converted into the general rudiments of all terminal organs of the inner ear, the so-called macula communis. Directly under it are located the rudiments of the eighth ganglia which either is formed from migrating cells of the auditory vesicle wall or has a mixed origin (Batten, 1958). Opposite the point of origin of the endolymphatic diverticulum, the lateral wall of the auditory vesicle forms a fold which grows into its cavity and divides the auditory vesicle into two parts: the upper, or the pars superior which forms an utricle and semicircular canal; and the lower, or the pars inferior forming the saccule and its

derivatives (lagena and acoustic papilla, and also the cochlear canal in birds and mammals). With the subdivision of the auditory vesicle into two parts, the macula communis is also divided into two sections: an upper and a lower which for a while remain interconnected by a ponticulum of characteristic epithelium; the latter is later replaced by the indifferent cubic epithelium of the auditory vesicle wall. The two separate neuroepithelial spots which are formed at the same time are located, respectively: the upper in the pars superior, and the lower in the pars inferior. Separate portions of the wall of the pars superior are thickened, and pocket-shaped protrusions are formed in these places. Their opposite walls adhere to one another and then these points of adhesion are resorbed. The semicircular canals are formed from the peripheral portions of these pockets. Both vertical canals originate from the general rudiment and their anterior ends discharge into the central portion of the utricle by means of the general crus communis or sinus superior utriculi. The other ends of these semicircular canals discharge directly into the utricle by means of their ampullae. The flat pocket-shaped protrusion from which the horizontal (lateral) semicircular canal is formed is usually established somewhat later. The final development of this canal and its ampullae is usually also retarded in relation to both vertical canals. The rudiment of the receptor organs of the utricular portion of the labyrinth lying in the pars superior gives rise to the utricular auditory spot, the macula utricularae and the ampullar crests of the anterior vertical and horizontal semicircular canals. The rudiment of receptor organs lying in the pars superior provides a process within and to the rear of the posterior ampulla, thus forming its auditory crest (crista ampullaris posterior). The remaining portion of this rudiment grows in length and in turn is subdivided into two rudiments: the smaller upper one, and the larger lower one. From the smaller one develops the saccular auditory spot, the macula sacculi; the larger lower one develops further and forms the rudiment of Corti's organ. In the lower vertebrates the auditory spot of the lagena (macula lagena) also develops from the external end of this rudiment. However the ontogenesis of individual auditory spots and crests has not been finally clarified to date (Alexander, 1926; Kolmer, 1927; de Burlet, 1934, et al.).

The distribution of nucleic acids (DNA and RNA), total protein and functional groups of protein molecules (SS, SH and COOH) during the development of receptor structures of the membranous labyrinth in all vertebrates which we studied generally changes identically. In individual representatives of one class or another, the enumerated chemical substances may differ chiefly by their concentration or changes in the distribution in individual structures at one stage or another. In the first stages of formation of the auditory vesicle and its ganglion, in all types of vertebrates studied the nucleic acids (RNA and DNA) and total protein are distributed more or less equally and give a reaction of average intensity. Electron microscope investigations conducted at this stage indicate great homogeneity of the cytoplasm in the cells of the auditory vesicle and the

underlying rudiments of the ganglion. It is possible to note only the presence of a large number of granules of the ribosome type in the cytoplasm and a moderate number of mitochondria, primarily of elongated shape with very densely distributed cristae and a dark matrix, i.e., the type which is characteristic for cellular elements with a predominating anaerobic type of exchange. Moreover, in the cytoplasm of these cells it is possible to see a significant number of rather large vesicles or vacuoli with very pale, almost transparent content. The plasmatic membrane of a cell at these stages is well defined. The round nuclei are surrounded by a clear, double-contoured membrane with well defined nucleoli and uniformly distributed chromatin in the form of small granules. The membranes of the endoplasmatic reticulum at this stage are completely absent.

The first sign of the beginning cellular differentiation is the change in form and size of the nucleolus and the intense increase in the concentration of nucleic acids, total protein and functional groups (SS, SH and COOH) of molecules contained in it. Nucleoli of the cells of the rudiment of the macula communis are increased in size and are shifted toward the periphery of the nucleus. The intensity of the reaction for nucleic acids (DNA and RNA), total protein and its functional groups (SS, SH and COOH) begins to increase. On electron microphotographs it is possible to see that the ribosomes on the side of the nucleolus are distributed nonuniformly; in the center of the nucleolus lumina are often formed from which traces of ribosomes stretch to the periphery of the nucleus. In the cytoplasm of such cells the formation of membranes of the endoplasmatic reticulum begins. The rather numerous mitochondria are distributed through the entire cytoplasm, but accumulations of them are observed primarily in the basal portions of the cell. /160

Upon subdivision of the cells of the rudiment into sensory and supporting cells, the structure and shape of their nuclei change first. Nuclei of supporting cells have denser and more crudely dispersed chromatin than the round nuclei of receptor cells. Differentiation of the cells is accompanied by a general drop in the intensity of reaction for RNA, total protein and its functional thiol and carboxyl groups. Even so, in the differentiating receptor cells the intensity of reaction for total protein is somewhat higher than in the neighboring supporting cells. The developing sensory hairs are distinguished initially by a very weak reaction for total protein which then increases somewhat; under electron microscope investigation this is explained by an increase in the general volume of the stereocilia and the concentration of the fine fibrous substance composing them. Upon final differentiation of a receptor cell, redistribution of RNA, total protein and its functional groups takes place in it. Thus in differentiated pitcher-shaped receptor cells of the organ of gravitation (utricle macula), RNA is basically distributed in the form of a granular accumulation in the basal portion, and in several animals even in the apical portion of the cell. Total protein and its carboxyl groups are most intensive in the apical portions of receptor cells, in which the most intensive reaction is ob-

served in their cuticle. Electron microscopically this corresponds to the stage involving development of the cuticle and the concentration of cytoplasmatic structures. As opposed to the utricular macula, receptor cells of Corti's organ in birds and mammals are characterized by a weak content and equal distribution both of RNA and of DNA. On the other hand, the total protein content in them is very great, the most intensive reaction being observed both in the cuticle and in the sensory hairs.

Differentiation of the ganglionic cells is characterized by the fact that RNA in them forms characteristic clumps, the so-called Nissl's substance, the distribution of which is specific for individual neurons and characterizes their functional state. Even carboxyl groups of protein reveal the same nature of distribution.

The oxidizing enzymes which we studied are originally manifested in all tissues of the rudiment and have a very high activity. They are localized in the mitochondria which are transferred into the cytoplasm of the cells during the process of differentiation. Originally the dense rod-shaped mitochondria are rounded, their cristae are distributed less densely and the matrix becomes more transparent. With the intensification of the processes of protein synthesis, mitochondria are distributed around the membrane of the endomembranous reticulum, i.e., directly around those places which are connected with protein synthesis. At the beginning of specific differentiation of the cells, when the formation of sensory hairs and cuticle takes place in the receptor cells, mitochondria are grouped in the apical part of the cell. With the formation of synapses with approaching nerve endings, some of the mitochondria are gathered in the basal part of the cell near the presynaptic membrane. The nerve endings forming synapses are also rich in mitochondria, which display a significant activity of oxidizing enzymes. The activity of CCO is significantly weaker in Corti's organ. In the inner ear of birds at the end of the embryonal period, CCO has a greater activity in the saccular macula and in the cristae and a lower activity in the utricle and in Corti's organ. High activity of CCO is observed in the ganglionic cells, in the vestibular and cochlear ganglia and in the ascending nerve fibers. /161

The appearance of specific cholinesterase in receptor structures of the membranous labyrinth corresponds to the period of nerve fiber ingrowth into the rudiments of receptor epithelium and its concentration on the synaptic surface; i.e., with the formation of synapses between receptor cells and nerve endings.

Thus the development of receptor formations of the membranous labyrinth of vertebrates, i.e., the organ of gravitation (utricle), organ of vibration (sacculus), auditory crests of the semicircular canals and Corti's organ, indicates that the direction of cytochemical changes in the process of embryonal development common to all classes of vertebrates is manifested in various receptor structures of the membranous labyrinth in different time periods, reflecting

the general regularity of the development of individual portions in phylogenesis. Thus during the earliest period the process of morphological and cytochemical differentiation begins and ends in the utricular macula (i.e., in the organ of gravitation), indicating its earlier preparedness for functioning in comparison with the other receptor formations of the inner ear. The development and differentiation of the vestibular part of the labyrinth in the higher vertebrates always anticipates the development of its auditory part. Moreover, the phylogenetically younger crista of the horizontal semicircular canal, which is still absent in the cyclostomata, is differentiated somewhat later than the cristae of both vertical semicircular canals. However, these regularities are sharply pronounced in the embryonal development of the membranous labyrinth of lower vertebrates (fishes, amphibians), and are somewhat less distinct in the higher vertebrates (reptiles, birds, mammals), which undoubtedly explains the presence of heterochromia in the development of the membranous labyrinth. In birds and mammals the temporal distinction is preserved at the beginning of differentiation of auditory spots and crests; however, the termination of their differentiation converges in time and the entire vestibular apparatus of birds and mammals terminates its differentiation as a whole. On the other hand, the differentiation of receptor elements of Corti's organ is separated not only in time but also in space, since the processes of differentiation begin at the base of the cochlea and somewhat later terminate on its summit. The differentiated auditory receptor cell is capable of perceiving sounds; however, the frequency acoustic perception evidently depends primarily upon the development of auxiliary structures of Corti's organ, which develops later than the receptor and ganglionic elements.

CONCLUSION

The task of this study was to trace the significance and behavior of a number of chemical, biologically active substances and the submicroscopic structures connected with them in the process of differentiation, maintenance of the structure and metabolism of definitive receptor elements even during the specific function. As it seems to us, the investigations which were conducted enable us to understand several regularities in the development of the structure and function of the receptor formations of the inner ear on the cellular and subcellular levels of organization, which determine the evolution of the function (Orbeli, 1958) of the inner ear at various levels. Simultaneously similar investigations may even have a certain practical value, providing materials for the comprehension and location of exchange processes in the receptor structures of the inner ear, which indicates the prospect of their regulation by means of active interference. /162

As is apparent from the given factual material, histochemical changes in the differentiating rudiments of the receptor epithelium have regularities and, to a significant degree, are similar in both the higher and lower vertebrates.

The general characteristics of the histochemical differentiation of receptor formations of the inner ear on bony fishes and representatives of the class Amphibia, i.e., the green toad and the grass frog, allow us to note the basic regularities in cytochemical differentiation in the lower vertebrates, namely:

(1) An increase, before the beginning of differentiation, in the concentration of nucleic acids, total protein and carboxyl groups of protein molecules not only in the cytoplasm, but also in the nucleolus, which often occupies a marginal position inside the nuclear membrane. These histochemical changes always precede the morphological indications of differentiation of the rudiments.

(2) Differentiation of the maculae and cristae in the lower vertebrates always begins in one central portion of the receptor formation, whereupon there are sharp cytochemical differences between the differentiated receptor cells of the central portion and the peripheral indifferent cells. In addition, a reverse correlation in the content of RNA and protein in these elements takes place, which evidently is connected with the earlier function of the differentiated cells of the central portion.

(3) Part of the receptor epithelium of lower vertebrates is poor in thiol groups, which evidently are little connected with the processes of cellular differentiation. However, in the differentiated eighth ganglion, the concentration of thiol groups is significantly higher than in the receptor epithelium, and their distribution in the process of differentiation of ganglionic cells signifi-

cantly changes, evidently indicating the direct participation of thiol groups in the differentiation of the ganglionic cells.

(4) Subsequent processes of cellular differentiation to a significant degree are connected with the redistribution of RNA in the apical and basal portions of the cytoplasm with simultaneous RNA impoverishment of the cytoplasm of supporting cells. Also, for differentiated receptor cells an increase in reaction to protein in the basal and subnuclear zones of the cytoplasm and in supporting cells is characteristic. In the sensory hairs it is very small. Their reaction to protein is increased toward the end of the differentiation. The terminal stages of differentiation of ganglionic cells are connected with the appearance of Nissl's substance in the cytoplasm, characterized by high concentration of RNA and protein.

The development of the inner ear of the higher vertebrates is distinguished by several characteristics which are morphologically expressed first in the exarticulation of a new section of the inner ear, i.e., the organ of hearing or the so-called Corti's organ. Moreover, one type of cylindrical receptor cell with bud-shaped nerve endings is split into two types; in addition to the already described cylindrical cell (Type II cell), there appears the pitcher-shaped Type I receptor cell with scyphoid nerve endings, encompassing the entire body of the cell and forming a huge synaptic surface with it.

The characteristics of the development of the inner ear receptor structure in higher vertebrates is most clearly apparent with the development of the mammalian inner ear. It is possible to enumerate them:

(1) While the individual anatomical parts of the inner ear are separated almost simultaneously, their cytochemical differentiation somewhat diverges in time, i.e., the same process which we encountered in the development of the inner ear of the lower vertebrates is repeated. Thus, first the macula begin to be differentiated; next the ampullar cristae and later the auditory portion of the inner ear. Parallel to morphological differentiation, a drop in the RNA concentration, total protein and its functional groups is observed, which before the beginning of functioning is changed by the process of accumulation of these substances. Cell differentiation is also accompanied by a redistribution of the chemical, biologically active substances, which is especially notable in the formation of the cuticle and sensory hairs in the process of the nerve fiber growth with the formation of a tigroid in the developing neurons of the eighth ganglion, etc.

(2) In contrast to the lower vertebrates, in the developing receptor formations of the vestibular apparatus in higher vertebrates the differentiation processes of receptor cells take place immediately in several portions of the maculae (or crista) and encompass relatively large portions of the organ. This is especially

sharply pronounced with the differentiation of the maculae in the tortoise, but it remains true even for birds and mammals. It is possible that this is connected with the simultaneous backing of the otolith membrane along almost the entire length of the macula and with the simultaneous formation of a large number of small otoconia. In such developing maculae and cristae a sharp difference between the differentiated and indifferent cells of one rudiment or the other is absent. This leads to the fact that the vestibular portions of the labyrinth of higher mammals is developed and differentiated as a whole, and those divergences which are observed with the cytological differentiation of the individual parts finally meet in a single process of organ differentiation, which in mammals terminates toward the third day of postnatal life.

(3) The auditory part of the labyrinth is differentiated in the developmental process by the fact that in it the differentiation of individual parts of the cochlear canal is separated not only in time but in space. While in the basal parts of the cochlear canal the differentiation of Corti's organ is practically completed, the summit of the cochlea appears in later stages of differentiation. Thus the processes which prepare Corti's organ for the perception of different sound frequencies do not begin at all simultaneously. The morphological differences in the development of the vestibular and auditory parts of the mammalian inner ear basically consist of the fact that in the vestibular organ, the development of receptor cells and supplementary structures takes place simultaneously. Therefore when the final differentiation of both types of receptor cells terminates in the newborn animal, the organ is completely ready for functioning. In Corti's organ of prematurely born mammals, on the other hand, the differentiation processes in the receptor structures are practically completely terminated at the moment of birth, but the differentiation process in the auxiliary structures of Corti's organ terminates only on the tenth day of postnatal life and only after this organ is ready for the perception of various sound frequencies, which corresponds with the histophysiological and physiological data. /164

(4) For the cytochemical differentiation of receptor structures of the inner ear in higher vertebrates it is characteristic that in addition to nucleic acids, total protein and enzymes, thiol functional groups take part in the differentiation of receptor elements, which indicates a significant change in their concentration and location during histogenesis.

(5) Histochemical investigations of glycogen showed that the glycogen appears in receptor structures of the inner ear during their differentiation. However, while in fishes and amphibia glycogen is distributed in the receptor cells themselves, in the vestibular apparatus of birds and mammals it is basically located in cells of the auxiliary structures beyond the bounds of the sensory epithelium of the auditory spots and crests. Corti's organ in birds, in this regard, adjoins the vestibular apparatus. On the

other hand, the auditory receptor cells of Corti's organ in mammals are distinguished by the unusual richness of glycogen, both at the embryonal and in definitive stages of development. The glycogen is contained also in the neurons of the vestibular ganglion but is absent in the neurons of the cochlear ganglion. The appearance of glycogen in the cytoplasm of the cells of the membranous labyrinth is connected with the development of dense rod-shaped mitochondria in them which are characteristic of cells with a pronounced anaerobic type of exchange. The presence of glycogen in the receptor cells of the vestibular apparatus in lower vertebrates evidently is connected with the characteristics of their metabolism.

Simultaneous cytochemical and electron microscope investigations of differentiation processes in the inner ear of a chick embryo indicated a continuous connection of the submicroscopic and cytochemical changes with the differentiation of receptor structures of the inner ear. While the changes in concentration and location of nucleic acids and proteins are closely connected with the development and distribution of ribosomes and elements of the endoplasmic reticulum, as well as changes in glycogen, the changes in respiratory enzymes are connected with the structure and redistribution of mitochondria and the enzyme activity of acetylcholinesterase; i.e., with the development of nerve endings and special synaptic structure.

Differentiation of ganglionic cells is characterized by the fact that RNA forms characteristic lumps in them, the so-called Nissl's substance, the distribution of which differs in individual neurons and partially characterizes their functional condition. The same nature of distribution in differentiated neurons is displayed by the carboxyl groups of proteins.

The cited regularities, revealing the connection between processes of differentiation and the change in content and location of chemically and biologically active substances in the structures of the inner ear is completely understandable from the point of view of current concepts about the interaction between the DNA nucleic code and the processes of cellular synthesis of chemical substances, in which RNA is a necessary intermediary. The synthesis of two types of tyrosinase of two neurospores may serve as an example of the direct interconnection of each protein to one gene (Horowitz and Fling, 1956). If the correlation between a gene change and a protein change is closely connected with the evolutionary process (Anfinsen, 1962), then this same correlation is undoubtedly connected with the processes of cell and tissue differentiation in the embryonic and histogenetic period, insofar as the development and differentiation, to a significant degree, depend upon the synthesis of proteins specific for the sequential stages of ontogenesis and of different tissues (Khesin, 1965). Examining the factors determining the synthesis of specific informational RNA, Khesin mentions the possibility of synthesis of special DNA molecules, designated for the formation of several informational RNA molecules (mRNA), necessary for a given stage of development. Khesin relates these spe-

cific DNA molecules to the cytoplasmic DNA discovered by I.B. Zbarskiy and L.S. Millman (1963), found in a large quantity in egg cells of amphibians and fishes, and sometimes exceeding the DNA content in the nucleus of the same egg cell by thousands of times. In addition, Astaurova (unpublished data cited by Khesin) showed that DNA from the eggs of the sturgeon are capable of serving as matrices even with the synthesis of DNA; i.e., they are capable of being duplicated.

Investigating informational RNA in early embryogenesis, A.S. Spirin, N.V. Belitsina and M.A. Aytkhozhin (1965) came to the conclusion that during embryogenesis beginning with the very earliest stages, protein synthesis takes place in the cytoplasm of the cells of the embryo, whereby the structures synthesizing the protein basically are the "heavy" ribosomes and polyribosomes, i.e., ribosome structures connected with mRNA. In addition, according to the data of these authors, polyribosome protein synthesizing structures of the cytoplasm, before the stage of the late blastula, contain matrix RNA synthesized not by the nuclei of the embryo but by a maternal nucleus in the process of ontogenesis. These ribosomes are reprogrammed by informosomes synthesized by the nuclei of the embryonic cells before gastrulation. During subsequent histogenesis, periodicity in reprogramming ribosomes can ensure a change in the synthesis of specific program proteins, determining the sequence in the passage of stages in the process of cellular differentiation and development (Spirin et al., 1965).

From this point of view, the investigations of P.V. Makarov and colleagues on the rhythmic changes in DNA and protein during the process of granulation are interesting, presenting a general regularity in the early embryogenesis of all groups of multicellular animals (Makarov, 1959). Hence, it is possible to refer to the data of S.A. Newfakh (1965) on the periodicity of genetic information transmission in the early development along the change "synthesis of RNA--process of morphogenesis".

The above data causes us to turn our attention to the embryonic induction continuously connected with the processes of differentiation which we do not investigate in the factual material we have presented, but which we must deal with briefly if we are to consider the processes of cellular differentiation from a biochemical point of view and relate them to influences of the genetic code.

Speaking of the concept of embryonal induction, Waddington (Waddington and Perry, 1960) indicates that this process, which is unclear at the present time, proceeds evidently according to the principle of controlling genes or activity. The latter, according to the two hypotheses available at the present time, may be performed either by means of activation of a system which is completely ready or, inversely, by means of depression; then this system is restrained by some internal agent which it is necessary to block. Waddington expresses the hypothesis that both depressions and activations must be preceded by an earlier process which can be called

"maturation". He considers that in the early stages of development the mechanism of protein synthesis is still "not ready" for either activation, or depression. Proteins are not components and are not capable of reacting until they become components. After completion of the competency period, they begin to synthesize a definite group of proteins.

It is especially interesting for us that competency can appear only at the last stage of development and that a definite period of maturation is necessary in order that the mechanism approaching synthesis be put into motion, the specific nature of which can be determined then in several different directions.

Such a morphogenetic and biochemical approach, in our opinion, helps us to understand those mechanisms which lie at the basis of the histochemical changes taking place during specific differentiation of receptor elements of the inner ear in vertebrates.

In discussing Khesin's report (1965), A.N. Studitskiy noted a difference in determining the essence of the differentiation concept from the biochemical and morphological point of view. While the biochemist defines differentiation as a process of change in protein syntheses "leading to a change in the cellular structure", from the point of view of a morphologist (according to Studitskiy's opinion) it is a visible structural change in the cell. However, these definitions do not greatly contradict each other in describing the essence of differentiation; it seems to us that the histochemical definitions of the differentiation process allow us to unite both these concepts into a single understanding of the differentiation process as a complex change in chemical conversions and a change in the synthetic processes of different chemically and biologically active substances (in the first place: nucleic acids, proteins and enzymes), directly connected with the substructural elements of the cell and therefore leading to a complication of its structure in comparison with the original form.

The differentiation of the receptor elements of the inner ear in vertebrates which we investigated is directly connected to the redistribution of chemically and biologically active substances in them, insofar as the specific nature of an impulse of the receptor cells of each analyzer under the influence of a definite stimulus is, in all probability, determined in the first place by one distribution or another of the substances (Vinnikov, 1959).

Cytochemical investigation of the inner ear in vertebrates at definitive stages of development showed that these histochemical characteristics which were found in separate classes of vertebrates in the embryogenesis of their inner ear are closely connected with the cytochemical, structural characteristics of receptor formations of the inner ear of definitive forms. Thus while in the process of embryonal development of receptor formations of the inner ear in one of the representatives of bony fishes (loach) we found a great

wealth of nucleic acids, total protein and carboxyl groups, which increases even more in neurons of the eighth ganglion, in adult representatives of the bony fishes (carp) the same characteristics in the cytochemical structure of definitive receptor structures of the inner ear and neurons of the eighth nerve are noted. In the embryonal development of receptor formations of the inner ear in the /167 frog, the absence of a single clear morphological characteristic of the developing receptor cells and the large variability of their cytochemical characteristics were noted. In definitive structures of their inner ear is also observed a large polymorphism of receptor elements and differences in the cytochemical characteristics of the maculae and cristae. Moreover, the thiol groups, which here occupy the same position as the carboxyl groups of protein molecules in the bony fishes, obtain a large specific gravity in the general chemism of receptor formations, especially in neurons of the eighth ganglion.

The hypothesis expressed in due course by Wersäll (1956) on receptor cells of the crista for definitive receptor cells of the mammalian inner ear was strongly confirmed in the literature, i.e., the concept that in the inner ear of mammals there are two types of receptor cells (pitcher-shaped and cylindrical) differentiated by body shape and by nerve ending structure. Both the internal and external hair cells of Corti's organ in mammals were later, with the investigation of Corti's organ of mammals proceeding from the innervation of their bud-shaped nerve endings, ascribed to the type of cylindrical cells. However, as it seems to us, with the contemporary stage of our knowledge of the substructural organization of hair cells, it is difficult to agree with this. The data given in the literary survey and submicroscopic structure of these cells, dealing with their forms, the forms of the cuticular lamina, the distribution and composition of sensory hairs, relations with the surrounding cells or fluids, and even submicroscopic data on the distribution of nerve endings upon them, and, finally, the submicroscopic structure of their cytoplasm, allow us to propose that these cells are new forms of evolution of a secondarily sensitive hair cell, which appears as a result of a functional evolution of the inner ear in the lower vertebrates, and it is more correct to view them as Type III and Type IV cells.

At the same time a second question, which we already raised, appears: are there differences in the cytochemical and substructural organization of pitcher-shaped and cylindrical hair cells in individual classes of vertebrates, i.e., do these cells change within the bounds of one cellular type in the process of phylogenesis? The investigations which we conducted showed that, cytochemically, these two types of receptor vestibular cells, best studied in the inner ear of mammals, are sufficiently clearly distinguished by the fact that for cylindrical receptor cells, a moderate concentration and equal distribution of nucleic acids, of protein, and of functional groups of protein molecules, with a certain increase in their concentration toward the apical end of the cell, were characteristic. A large concentration of protein and its functional groups is ob-

served in the cuticle of these cells. The pitcher-shaped receptor cells are differentiated from the cylindrical ones by the significantly greater high concentration of protein and its functional groups, which are also richly represented in their cuticle. But the fundamental cytochemical difference between these two types of receptor cells appears in the distribution of nucleic acids. In the pitcher-shaped receptor cells, in addition to the equal distribution of RNA giving a comparatively weak reaction in the basal part of the cell under the nucleus, there is a very bright, cap-like accumulation of granular RNA.

The cylindrical receptor cells, or Type II cells, of the vestibular apparatus of mammals, according to the electron microscope investigation of Wersäll, et al., (Wersäll, 1956, 1960; Wersäll and Hawkins Jr., 1962; Engström and Wersäll, 1958; Friedmann, 1962, 1963), can also, according to our investigations (Vinnikov, Gazenko, Titova et al., 1963b), contain a large number of equally distributed particles of moderate electron density in their cytoplasm; an insignificant number of α -cisterns and smooth membranes corresponding to the distribution of Golgi's apparatus are often found in their sub- /168 nuclear zones. There are no individual accumulations of mitochondria in them. Thus, the equality of distribution which we noted and the relative concentration of chemical biologically active substances find complete confirmation and explanation in the ultrastructural organization of these receptor elements.

For the pitcher-shaped receptor vestibular cells, or Type I cells, according to our data and the literary data (Wersäll, 1956; Engström and Wersäll, 1958; Friedmann, 1962, 1963; Vinnikov, Gazenko, Titova et al., 1963a), the presence of a large number of different organoids, intracellular membranous systems (α - and ν -cisterns) and mitochondria, which form accumulations both in the apical and in the basal portions, is characteristic. In the basal portions of these cells there are 1-8 membranes of α -cisterns of the granular endoplasmatic reticulum. The γ -cisterns, bounded by smooth membranes, are located in the supranuclear zone, evidently forming Golgi's apparatus. This pattern of ultrastructural organization of the pitcher-shaped receptor cells of mammals completely corresponds with the cytochemical data which we obtain and explains the large concentration of chemical biologically active substance in their cytoplasm and also the location of a granular accumulation of RNA in the subnuclear zones.

Besides these two types of cells, in Corti's organ of mammals, first in the phylogenesis of the inner ear there appear two types of auditory receptor cells, namely the internal and external receptor (hair) cells which, according to the accepted classification, are usually ranked with the cylindrical receptor cells, uniting the cylindrical receptor cells of birds and mammals under this designation as well as the cylindrical auditory receptor cells of birds and the external and internal receptor cells of Corti's organ of mammals. In all these receptor cells there is only one common

characteristic: all of them are innervated by nerve fibers proceeding from nerve plexuses and forming bud-shaped nerve endings at their base. However, the hair cells of Corti's organ of mammals are distinguished so much by significant ultra-structural and cytochemical characteristics that this hardly allows them to be united not only with the cylindrical receptor cells of the lower vertebrates but even with the cylindrical hair cells of Corti's organ of birds.

The internal and external hair cells are primarily distinguished from one another by their shapes: while the external hair cells have a regular cylindrical shape, the internal hair cells, because of their irregular body shape, are somewhat similar to the pitcher-shaped vestibular receptor cells.

A still greater difference between the auditory and vestibular receptor cells is observed in the structure of the sensory hairs. Both the internal and external hair cells of mammals are distinguished from all other receptor cells of the inner ear of vertebrates by the fact that the bundles of their sensory hairs consist only of immobile stereocilia; i.e., the mobile kinocilium with its characteristic fibrillar apparatus is absent. Of the kinocilium there only remains the basal corpuscle located in the cytoplasm of external and internal hair cells near their apical surface, beside the cuticle but in a portion of cytoplasm free from it. Its location relative to the bundle of stereocilia is polar, as is the location of kinocilia in the rest of the receptor cells of the acoustical lateral system. The presence of this basal corpuscle lacking kinocilia in the hair cells of Corti's organ in mammals was first described by Engström, Ades and Hawkins (1962). The absence of kinocilia distinguishes the external and internal hair cells of mammals not only from the vestibular receptor elements but also from the auditory cells of Corti's organ of birds. The bundles of sensory hairs contain not only immobile stereocilia, but even a mobile kinocilium with the characteristic fibrillar apparatus. /169

However, differences in the structure of sensory hairs are evident even among the internal and external hair cells. They concern both the location of stereocilia on the summit of the internal (2 rows) and external (3-5 rows) hair cells and in their thicknesses, i.e., stereocilia of the external hair cells are more than twice as thick as the stereocilia of internal hair cells (Engström, 1951; Iurato, 1961). The most interesting fact is that the number of stereocilia and the length of the bundle on the external hair cells regularly change, gradually increasing toward the base of the cochlea. There are also differences between the internal and external hair cells in the structure and distribution of nerve endings upon them.

According to the data of a series of electron microscope investigations (Engström and Wersäll, 1958a, 1958b; Engström, 1960; Iurato, 1961), the cytoplasm of external hair cells of Corti's organ

in its apical portion is rich in osmophyle granules and portions of membrane. In the basal part there is a large number of α -cisterns, bordered by the granulated membranes characteristic of cells with a high protein metabolism (Wersäll et al., 1961). The rest of the cytoplasm is thinly granulated, and mitochondria are scattered in it. The cytoplasm of external hair cells is distinguished by the presence of a large number of dense and interrupted membranes. The latter stretch in 6-7 layers along the vertical wall of the external hair cell accompanied by rod-like mitochondria located in 1 row. Their cytoplasm is distinguished by the transparency of the matrix and abundance of ribosomes. The nucleus of an external hair cell has a karyoplasm of moderate electron density and granules of chromatin.

Significant differences between internal and external hair cells are observed also in their cytochemical characteristics.

With a cytochemical investigation of Corti's organ it is possible to discover in them several differences in the distribution and concentration of chemical biologically active substances. Thus, in the internal hair cells in comparison with the external ones there are observed equal concentrations and distribution of RNA in their cytoplasm, but a somewhat smaller concentration of DNA in the chromatin of the nucleus. Internal hair cells are somewhat richer in protein and thiol groups, and a concentration of the substances in the subnuclear zone, absent in the external hair cells, is observed in their cytoplasm. But there is one cytochemical characteristic which distinguishes receptor cells of Corti's organ of mammals from their vestibular receptor cells; i.e., the wealth of their sensory hairs in total protein, thiol groups of protein molecules and, what is especially important, specific cholinesterase. A still greater cytochemical difference in these two types of receptor cells of Corti's organ is the absence of glycogen in the internal hair cells with an exceptional wealth of glycogens in the external hair cells. From the description of the ultrastructural organization of the cytoplasm of these cells it becomes comprehensible why the internal hair cells are so much richer in protein and functional groups of protein molecules and in this regard approximate the pitcher-shaped vestibular receptor cells, in which one observes a similar distribution both of the ultrastructural components of the cytoplasm and of the chemical biologically active substances. /170

Moreover, the data given here indicates that in Corti's organ the "young" (in an evolutionary regard), i.e., first-appearing in a phylogenetic process in the mammals, external and internal hair cells, distinguished from one another by their microscopic structure, show significant cytochemical differences appearing as the same precise characteristic as the characteristics of their structure and distribution in the system of cellular elements of Corti's organ.

From our factual data it is possible to draw the conclusion

that the most stable are the cylindrical receptor cells, which have the same structure in the inner ear as in the lateral line organs (Flock, 1964; Aronova, 1967). In addition, it is necessary to keep in mind that these cells of the lower vertebrates served as the original material for the development in the higher animals of the pitcher-shaped Type I receptor cells. Unfortunately, the response to this question is hindered due to the scarcity of available material. However, if we compare the submicroscopic structure of the cylindrical receptor cells of the skate (Löwenstein et al., 1964) with the structure of cylindrical cells of mammals (cf. p. 16), then the conclusion is suggested that the submicroscopic structure of the cylindrical receptor cell of the skate is significantly more complex than the cylindrical vestibular receptor cell of mammals and has a number of common features with the pitcher-shaped vestibular receptor cell. We come to the same conclusion from a comparison of the cytochemical characteristics of these cells on the basis of our data. If we remember the polymorphism of cylindrical receptor cells in the inner ear of the frog, then it is possible to express the hypothesis that cylindrical receptor cells of fishes and amphibians are the original type which sometime during the transition to reptiles were separated into two types of receptor elements in higher vertebrates.

As far as the differences in the cytochemical and submicroscopic vertebrates is concerned, from the factual material which we have given, the cytochemical differences in them are sufficiently clear. If we compare the pitcher-shaped receptor cells of the tortoise, birds (rooster, pigeon) and mammals (cat, guinea pig), it is possible to note the following characteristics and the distribution of nucleic acids in them. In the tortoise, nucleic acids, in particular RNA in the pitcher-shaped receptor cells, have a greater concentration than in the amphibians, but within the bounds of a cell they are located completely equally. In birds, in the pitcher-shaped cell there are two clear accumulations of granular RNA which are located above and below the nucleus. With electron microscope investigations of the pitcher-shaped receptor cells of the utricular macula of the pigeon in our laboratory, small concentrations of α -cisterns in the cytoplasm of these cells above and below the nucleus were found. In mammals, as was already mentioned, such granular accumulations of RNA are observed only in the subnuclear zone of the cytoplasm and are also connected with the accumulation here of α -cisterns (Vinnikov, Gazenko, Titova, Osipova et al., 1963).

The comparison of our cytochemical patterns with those available in the literature and the data received in our laboratory, according to the ultrastructural organization of receptor cells of the membranous labyrinth of mammals, allows us to draw the conclusion that the cytochemical characteristics of their organization find their confirmation, and are closely connected with, their ultrastructural organization. In addition it appears to be the rule that these hereditarily strengthened changes must be connected with a change in the genetic code. /171

Data concerning the fact that the variously functioning cells are distinguished from one another not only by their morphological structure but also by their cytochemical characteristics is confirmed by a series of investigations of other organs and tissues (Roskin and Shornikova, 1953; Roskin et al., 1954; Roskin and Struve, 1959; Portugalov and Yakovlev, 1958; Portugalov, 1959; Levinson, 1958, 1961; Sisakyan and Filipovich, 1959).

Thus the structure and metabolism of all receptor cells of the inner ear in a state of relative rest are maintained by the same chemical, biologically active substances; however, the nature of their distribution is characteristic for each type of cell.

As far as the changes in the chemical biologically active substances, basically RNA and proteins in various functional states and with a functional load of the cell is concerned, they were investigated in a number of biochemical and then in cytochemical works dedicated chiefly to the investigation of nerve tissue (Hyden, 1943, 1955; Hochberg and Hyden, 1949; Caspersson, 1950; Brashe, 1950, 1957; Palladin, 1952; Vladimirov, 1953; Kreps et al., 1954; Kreps and Chenykayeva, 1955; Smirnov, 1955; Einarson, 1957; Vinnikov and Titova, 1957, 1958a, 1958b, 1958c, 1961; Danilova, 1958; Levinson, 1958, 1961; Brodskiy and Nechayeva, 1959; Vinnikov, Titova et al., 1960; Lukashevich, 1960; Bronshteyn and Krestinskaya, 1961; Yakovlev et al., 1961, and others); and their data do not contradict ours which were obtained from an investigation of the inner ear.

The factual material which we have presented indicates the changes in concentration and location (redistribution) of a number of chemical biologically active substances in the receptor cells of the inner ear with the action of adequate stimulation upon them. In addition, we observe a number of sequential changes in the ribonucleic acid total protein, functional groups of protein molecules, glycogen and a number of respiratory enzymes and the specific enzyme of nerve conductivity, i.e., acetylcholinesterase. The connection of chemical biologically active substances noted in the process of histogenesis and in the intact receptor cell with definite submicroscopic structural elements of the cell allowed us to reveal even in a functioning receptor cell a number of structural shifts of its submicroscopic components corresponding to the discovered cytochemical changes. Thus the output of nucleolic RNA into the cytoplasm, which we discovered cytochemically, is connected with the output from the nucleus, through the pores of the nuclear membrane, of nucleolic ribosomes which settle on the spiral-shaped membranes of the endoplasmic reticulum formed in the basal part of the cell. Precisely the same changes of enzymatic succindehydrogenase activity and its redistribution in a functional receptor cell and in the nerve ending are connected with the removal of mitochondria into synapse regions, which often terminate in a close contact of separate mitochondria with the presynaptic membrane ("application" of mitochondria), leading to an increase in the number of contacting membranes.

It is necessary to note that the output of ribosomes of the nucleolus is observed in receptor cells of the utricle both in a condition of relative rest and, evidently, also takes place during processes of differentiation. This output of RNA takes place spontaneously with a certain constant rhythm. Experimental functional activities accelerate this rhythm of nucleolar RNA output appearing as an activating mechanism for a whole number of synthetic protein processes, with the aid of which cellular respiration takes place after increased functioning.

Similar cytochemical and substructural changes of functioning /172 receptor cells of the inner ear are completely regular and are found in representatives of all classes of vertebrates. The degree of their appearance is completely defined by the strength and duration of activity.

Summarizing all that was presented above, we can draw the conclusion that these chemical, biologically active substances which appear to be basic components of the substructural organization of a cell participate both in the processes of its metabolism and specific functioning, at the same time actively participating even in its cytological differentiation in the process of embryonal development.

ILLUSTRATIONS (1-73)

List of Designations on the Figures

A	- Ampulla	DC	- Deiter's Cells	/175
AAVC	- Ampulla of Anterior Ver- tical Canal	DG	- Diffuse Glycogen	
AHC	- Ampulla of Horizontal Canal	DKC	- Dark Cells	
AO	- Average-sized Otolith	E	- Embryo	
APVC	- Ampulla of Posterior Ver- tical Canal	EC	- Epithelial Cell	
AV	- Auditory Vesicle	ECC	- External Cell Columns	
BC	- Basal Corpuscle	EHC	- External Hair Cells	
BK	- Backing of Kinocilium	ESI	- Epithelium of Spiral Inci- sure	
BM	- Basal Membrane	ESL	- Epithelium of Semilunar Lamina	
BNF	- Bundle of Nerve Fibers	G	- Ganglion	
BPM	- Bundle of Parallel Mem- branes	GA	- Golgi's Apparatus	
BSC	- Basal Part of Supporting Cell	GG	- Glycogen Granules	
BSH	- Bundle of Sensory Hairs	GME	- Granulated (rough) Membranes of Endoplasmatic Reticulum	
BSM	- Basilar Membrane	HC	- Hensen's Cells	
BV	- Blood Vessel	HE	- Head of Embryo	
C	- Crista	HMC	- Homogeneous Cells	
CAV	- Cavity of Auditory Ves- icle	HSS	- Helmet-shaped Space	
CBM	- Cords of Basilar Mem- brane	HYC	- Hyaline Cells	
CC	- Claudius' Cells	ICC	- Internal Cell Columns	
CCN	- Cochlear Canal	IHC	- Internal Hair Cell	
CCO	- Cavity of Corti's Organ	K	- Kinocilium	
CCR	- Complex Crista	L	- Lagena	
CE	- Centriole	LC	- Light Cells	
CF	- Central Fibrils	LM	- Lagena Macula	
CG	- Cochlear Ganglion	LO	- Large Otolith	
CHC	- Crista of Horizontal Canal	M	- Macula	
CNT	- Connective Tissue	MC	- Mesenchymal Cell	
CP	- Capsule	MCO	- Macula Communis	
CR	- Ciliar Rod	MER	- Membranes of Endoplasmatic Reticulum	
CS	- Cell Satellites	MTA	- Mitochondria	
CSL	- Cells of Spiral Limb	MV	- Microvilli	
CSU	- Cell Summit	N	- Nucleus	
CT	- Cuticle	NC	- Nerve Cup	
CU	- Cupula	NE	- Nerve Endings	
CUT	- Cavity of Utriculus	NF	- Nerve Fibers	
CVC	- Crista of Vertical Canal	NL	- Nucleolus	
CY	- Cytoplasm	NM	- Nuclear Membrane	
D	- Desmosome	NR	- Neuron	
		O	- Otolith	
		OM	- Otolith Membrane	
		PF	- Protofibrils	

PM	- Plasmatic Membrane	RUMC	- Rudiment of Utricular Macu-
PNM	- Pores of Nuclear Mem-		la and Crista
	brane	S	- Sacculus
PPM	- Protuberance of Plas-	SC	- Simple Crista
	matic Membrane	SG	- Secretory Granules
PRF	- Peripheral Fibrils	SH	- Sensory Hairs
PS	- Prominentia Spiralis	SL	- Semilunar Lamina
PSM	- Pre-synaptic Membranes	SN	- Segmented Nucleus
PTM	- Post-synaptic Membrane	SNE	- Scyphoid Nerve Endings
R	- Ribosomes	SO	- Small Otolith
RC	- Rudiment of Crista	SPC	- Supporting Cell
RCO	- Rudiment of Corti's	ST	- Stereocilia
	Organ	TM	- Tectorial Membrane
RM	- Reissner's Membrane	TV	- Tegmentum Vasculosum
RPK	- Roots Proceeding from	UT	- Utriculus
	Kinocilium	UM	- Utricula Macula
RRC	- Receptor Cells	V	- Vacuoli
RRC I	- Type I Receptor Cells	VG	- Vestibular Ganglion
RRC II	- Type II Receptor Cell	VS	- Vascular Stria
RRE	- Receptor Epithelium	WAV	- Wall of Auditory Vesicle
RTM	- Reticular Membrane	Y	- Yolk

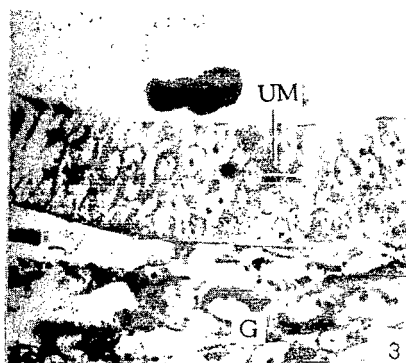
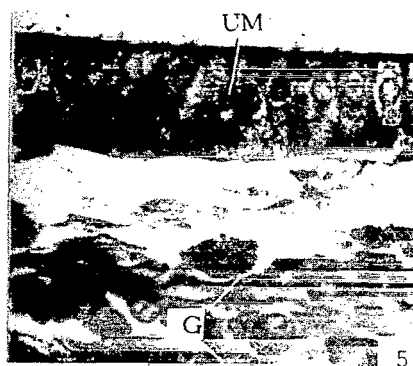
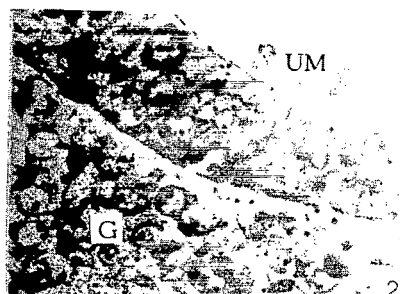
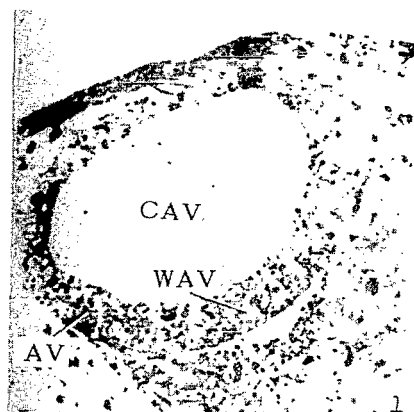


Fig. 1. Structure of the Auditory Vesicle and Ganglion of the White Sturgeon Embryo Before Pecking Out. Treated for Carboxyl Groups According to Barrnett and Seligman; ob. $\times 20$, oc. $\times 7$.

Fig. 2. Same as Fig. 1. Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 3. Structure of the Saccular Macula and Underlying Ganglion of the Prelarva of a White Sturgeon 6 Days After Pecking Out. Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 4. Saccular Macula of the Passively Drifting Larva of the White Sturgeon 8 Days After Pecking Out. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 5. Saccular Macula and Ganglion of an Actively Feeding Larva of the White Sturgeon 15 Days After Pecking Out. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. $\times 7$.

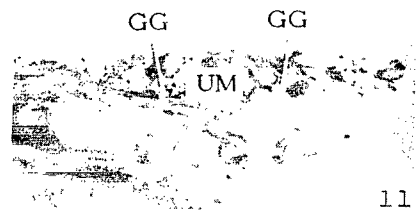
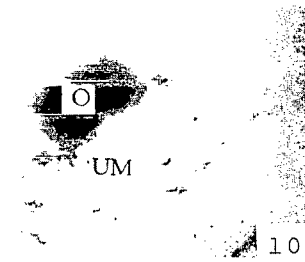
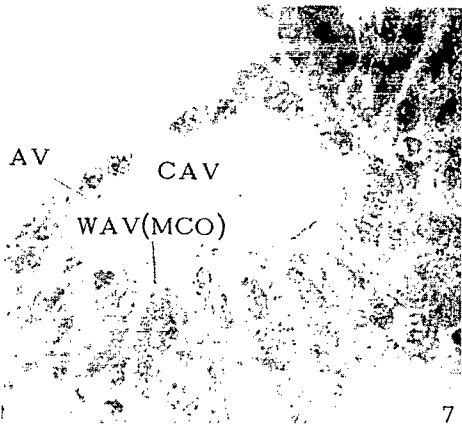
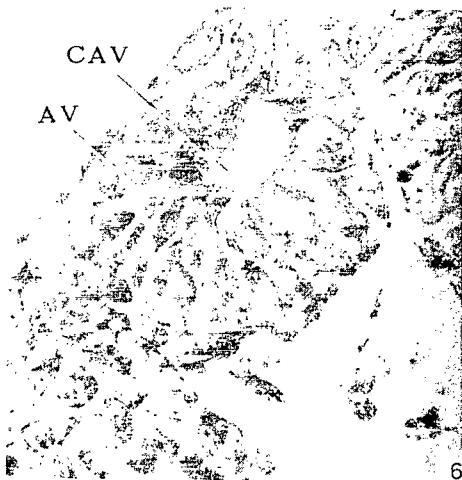


Fig. 6. Structure of the Auditory Vesicle and Adjacent Ganglion of the Loach at the Third Roe Stage. Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 7. Same as Fig. 6. Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 8. Lagena Macula of the Loach Larva After the Beginning of Active Feeding. Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 9. Ganglion of the Loach Larva the 4th Day After Beginning Active Feeding. Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 10. Otolith of the Utricular Macula of a Passively Drifting Loach Larva. Treated for Glycogen According to Lilly; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 11. Utricular Macula of the Loach Larva at the Beginning of Active Feeding. Treated for Glycogen According to Shabadash; imm. ob. $\times 60$, oc. $\times 10$.

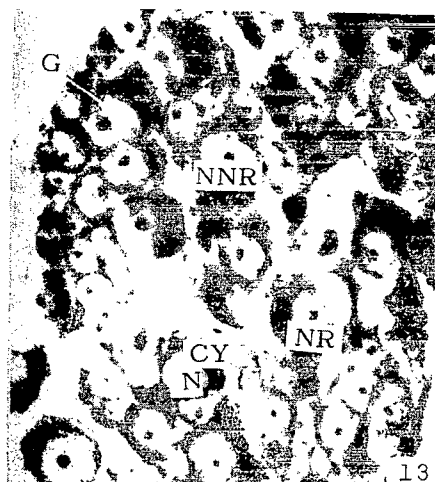
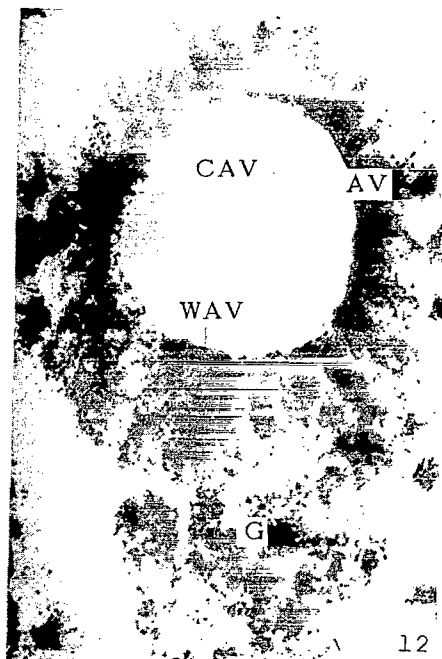


Fig. 12. Structure of the Auditory Vesicle of a Frog Tadpole (stage 18-19). Fixation with Zenker-Formol, Staining with Ferric Hemotoxaline According to Yasvoyn; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 13. Ganglion of the Frog Tadpole (stage 21-22). Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 14. Crista and Ganglion of the Frog Tadpole (stage 27-28). Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 15. Macula of the Frog Tadpole Before the Beginning of Metamorphosis. Treated for Nucleic Acids According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 16. Utricular Macula of the Frog Tadpole (stage 27-28). Treated for Glycogen According to Shabadash; imm. ob. $\times 60$, oc. $\times 7$.

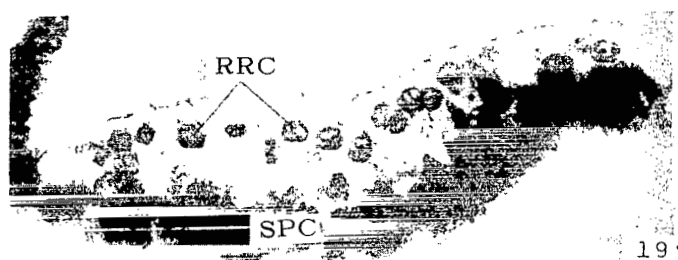
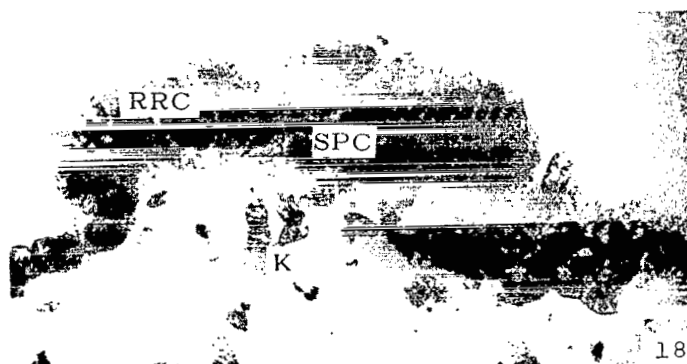
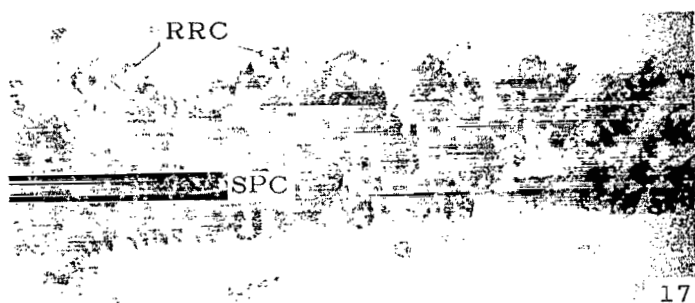


Fig. 17. Macula of a Tortoise Embryo 14 mm in Parieto-Coccygeal Length. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 18. Crista of the Tortoise Embryo 14 mm in Parieto-Coccygeal Length. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 19. Lagena Macula of a Tortoise Embryo 18 mm in Parieto-Coccygeal Length. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 20. Ganglion of a Tortoise Embryo 18 mm in Parieto-Coccygeal Length. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

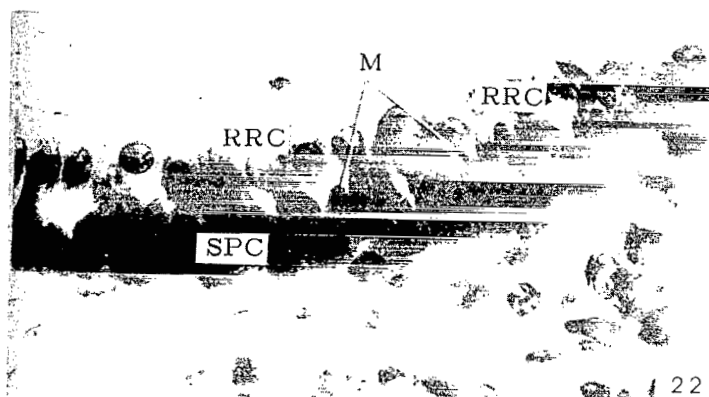
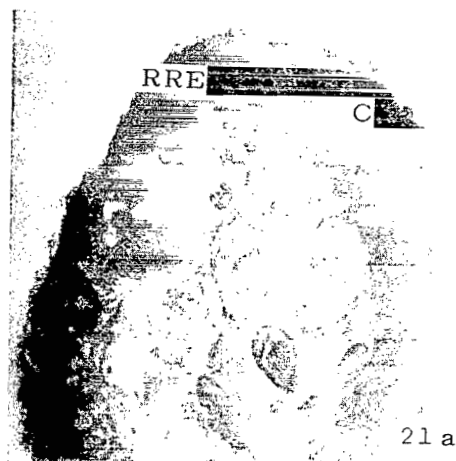


Fig. 21. Crista of Tortoise Embryo 18 mm in Parieto-Coccygeal Length. (a) Treated for Thiol Groups According to Yakovlev and Nistratova; imm. ob. $\times 60$, oc. $\times 7$; (b) Treated for Sulfhydryl Groups According to Yakovlev and Nistratova; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 22. Macula of the Tortoise Embryo 23 mm in Parieto-Coccygeal Length. Treated for Nucleic Acids According to Einarson; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 23. Ganglion of the Tortoise Embryo 23 mm in Parieto-Coccygeal Length. Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. hom. 4.

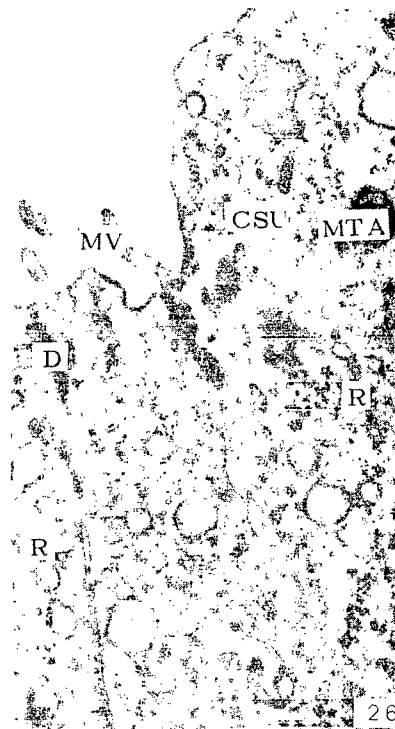
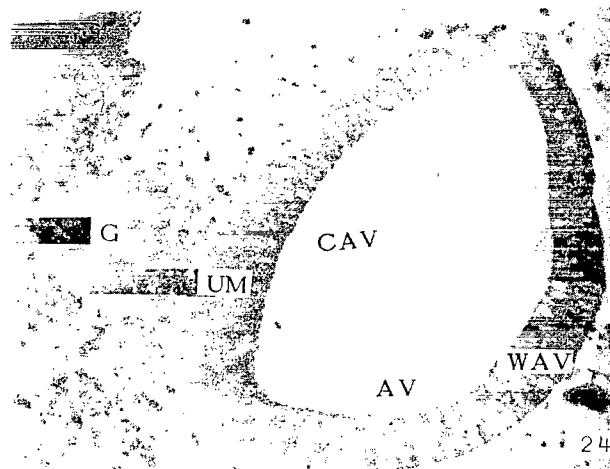
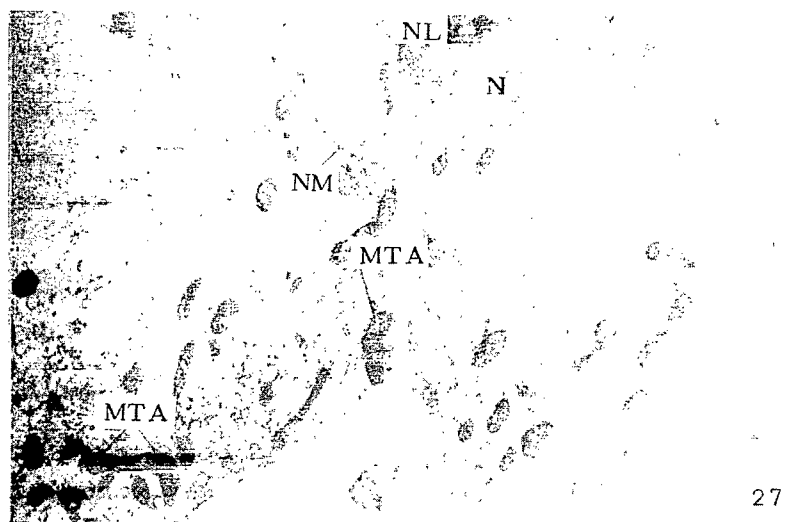


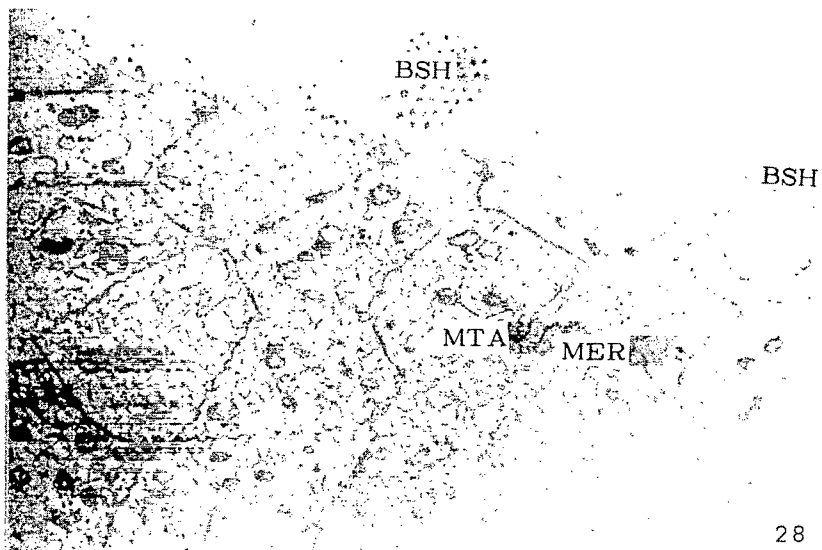
Fig. 24. Structure of the Auditory Vesicle of a Chick Embryo of 4 Days Incubation. Fixation According to Shampi, Staining with Ferric Hematoxyline According to Yasvoyn; imm. ob. $\times 60$, oc. $\times 7$.

Fig. 25. Macula Communis of a Chick Embryo of 4 Days Incubation. Electron Microphotograph, mag. 5600.

Fig. 26. Apical Portion of the Cells of the Macula Communis of a Chick Embryo of 4 Days Incubation. Electron Microphotograph, mag. 30,000.



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Fig. 27. Basal Portion of the Cells of the Macula Communis of a Chick Embryo of 4 Days Incubation. Electron Microphotograph, mag. 10,200.

Fig. 28. Receptor Epithelium of the Utricular Macula of a Chick Embryo of 11 Days Incubation. Electron Microphotograph, mag. 6700.

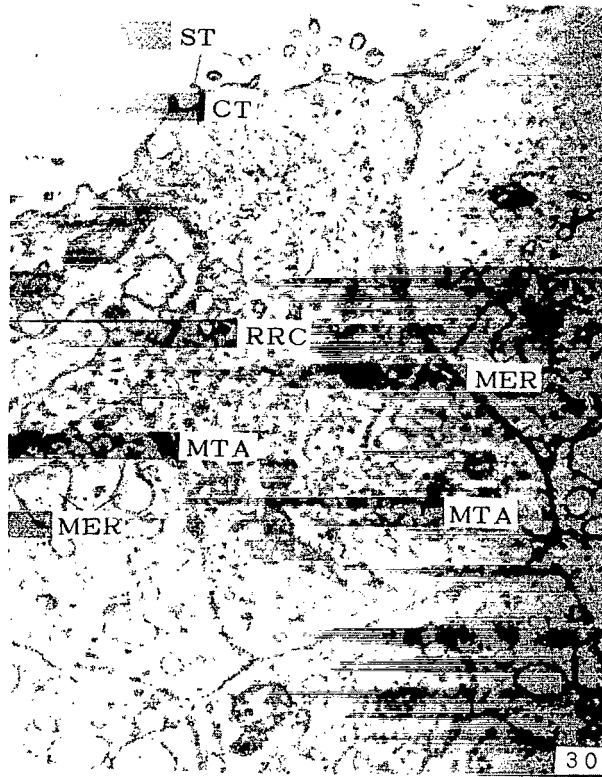


Fig. 29. Surface of the Utricular Macula of a Chick Embryo of 11 Days Incubation. Electron Microphotograph, mag. 35,500.

Fig. 30. Upper Portion of the Receptor Epithelium of the Utricular Macula of a Chick Embryo of 11 days Incubation. Electron Microphotograph, mag. 11,000.

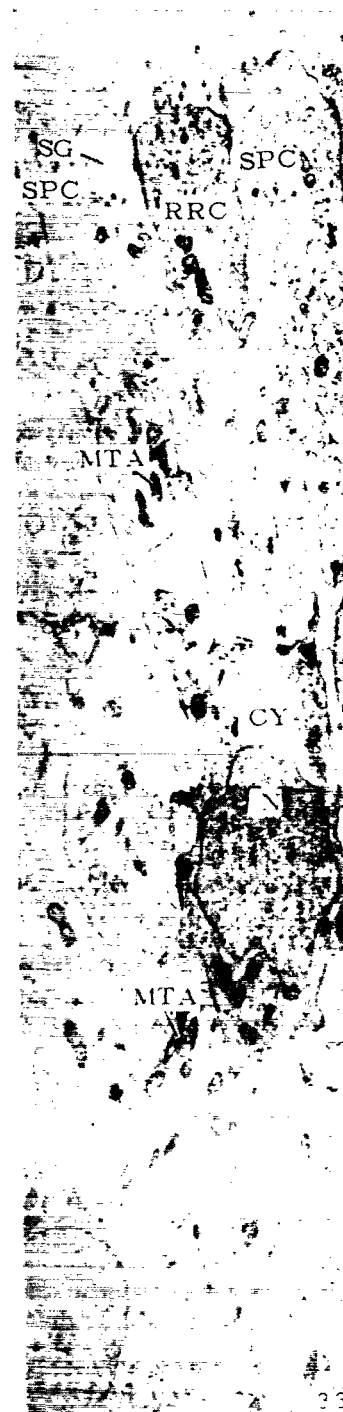
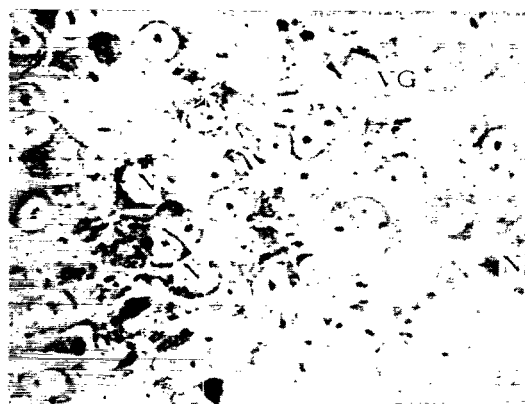


Fig. 31. Basal Membrane of the Receptor Epithelium of the Utricular Macula of a Chick Embryo of 11 Days Incubation. Electron Microphotograph, mag. 56,000.

Fig. 32. Vestibular Ganglion of a Chick Embryo of 12 Days Incubation. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. hom. 6.

Fig. 33. Receptor Epithelium of Utricular Macula of a Chick Embryo of 15 Days Incubation. Electron Microphotograph, mag. 5600.

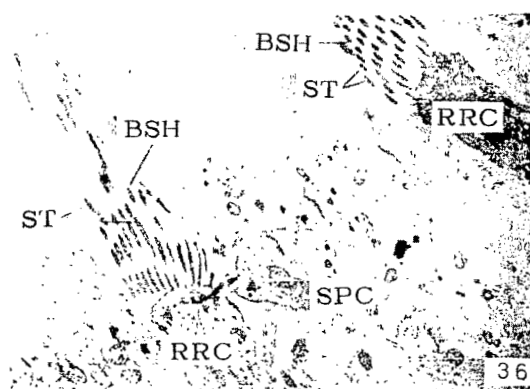
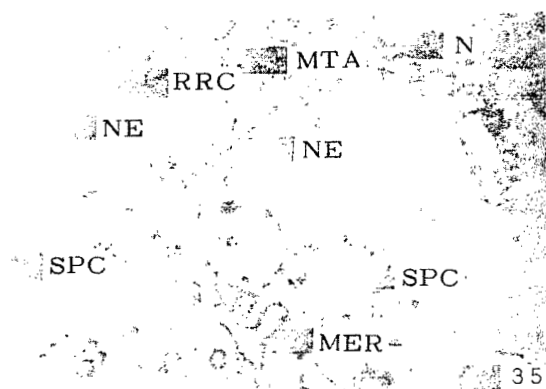
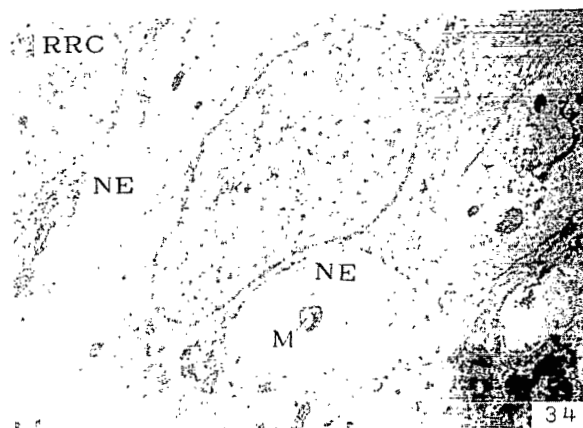


Fig. 34. The Same Stage as in Fig. 33. Nerve Ending on a Dark Receptor Cell in the Utricular Macula. Electron Microphotograph, mag. 7000.

Fig. 35. The Same Stage as in Fig. 33. Nerve Endings at the Base of a Dark Receptor Cell in the Utricular Macula. Electron Microphotograph, mag. 23,000.

Fig. 36. The Same Stage as on Fig. 33. Upper Portion of the Receptor Epithelium of the Utricular Macula. Electron Microphotograph, mag. 3200.

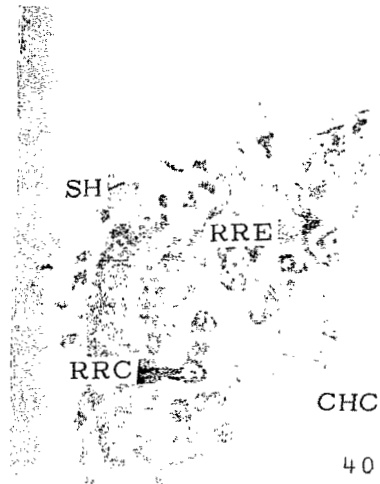
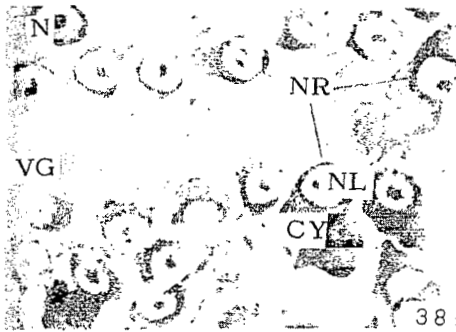
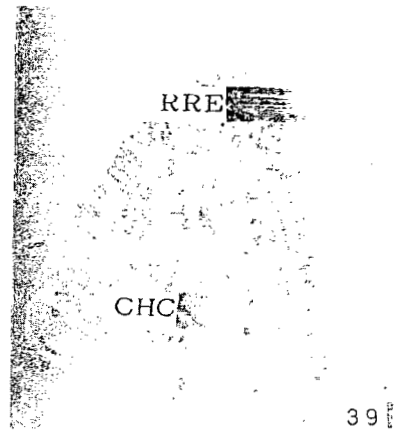
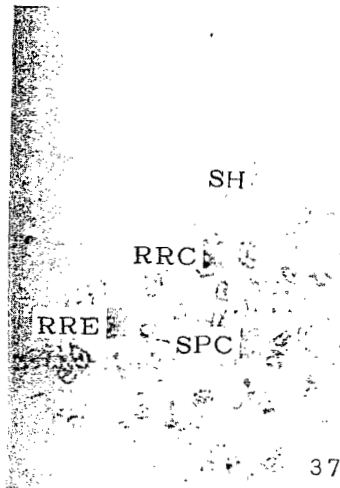


Fig. 37. Lagna Macula of a Chick Embryo of 15 Days Incubation. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. hom. 6.

Fig. 38. Vestibular Ganglion of a Chick Embryo of 15 Days Incubation. Treated for Nucleic Acid According to Barrnett and Seligman; imm. ob. $\times 60$, hom. 6.

Fig. 39. Crista of the Horizontal Semicircular Canal of a Chick Embryo of 15 Days Incubation. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 20$, oc. hom. 6.

Fig. 40. Structure of the Receptor Epithelium at the Summit of the Same Crista as on Fig. 39. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. hom. 6.



Fig. 41. Complex Crista of the Semicircular Canal of a Chick Embryo of 19 Days Incubation. Treated for Nucleic Acid According to Einarson; ob. $\times 10$, oc. hom. 6.

Fig. 42. The Same Stage as on Fig. 41. Receptor Epithelium at the Summit of the Same Crista. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. hom. 6.

Fig. 43. Semilunar Lamina of the Vertical Ampulla of a Chick Embryo of 19 Days Incubation. Treated for Nucleic Acid According to Einarson; imm. ob. $\times 60$, oc. hom. 6.

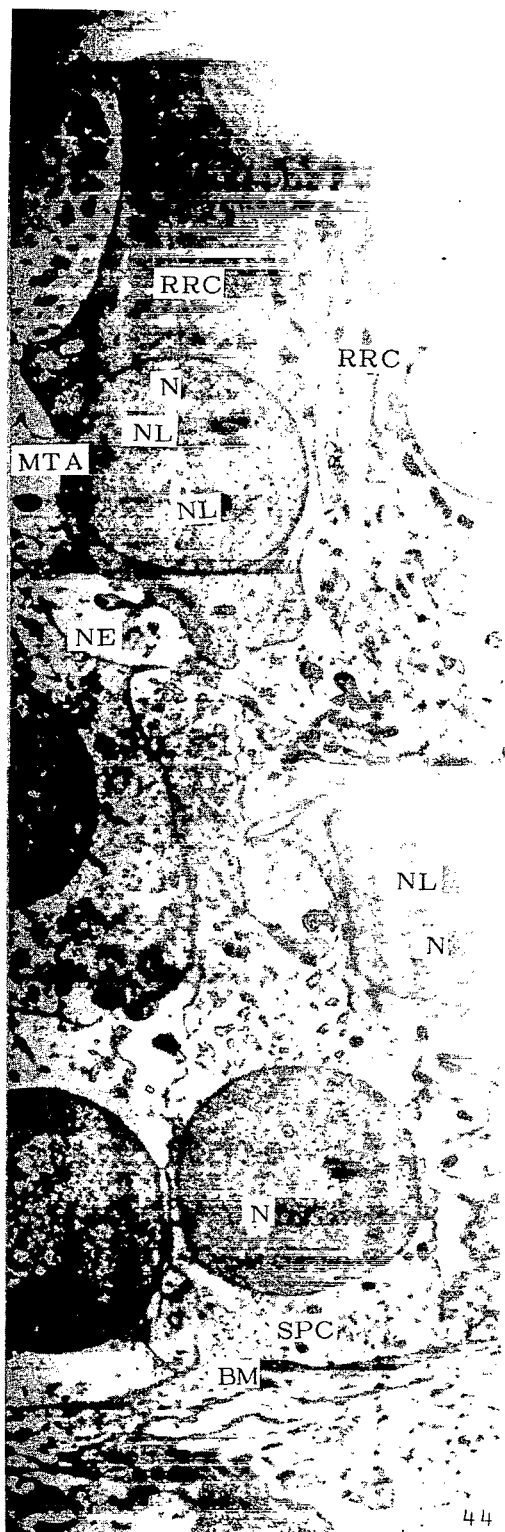


Fig. 44. Receptor Epithelium of Utricular Macula of a Chick Embryo Before Hatching. Electron Microphotograph, mag. 7000.

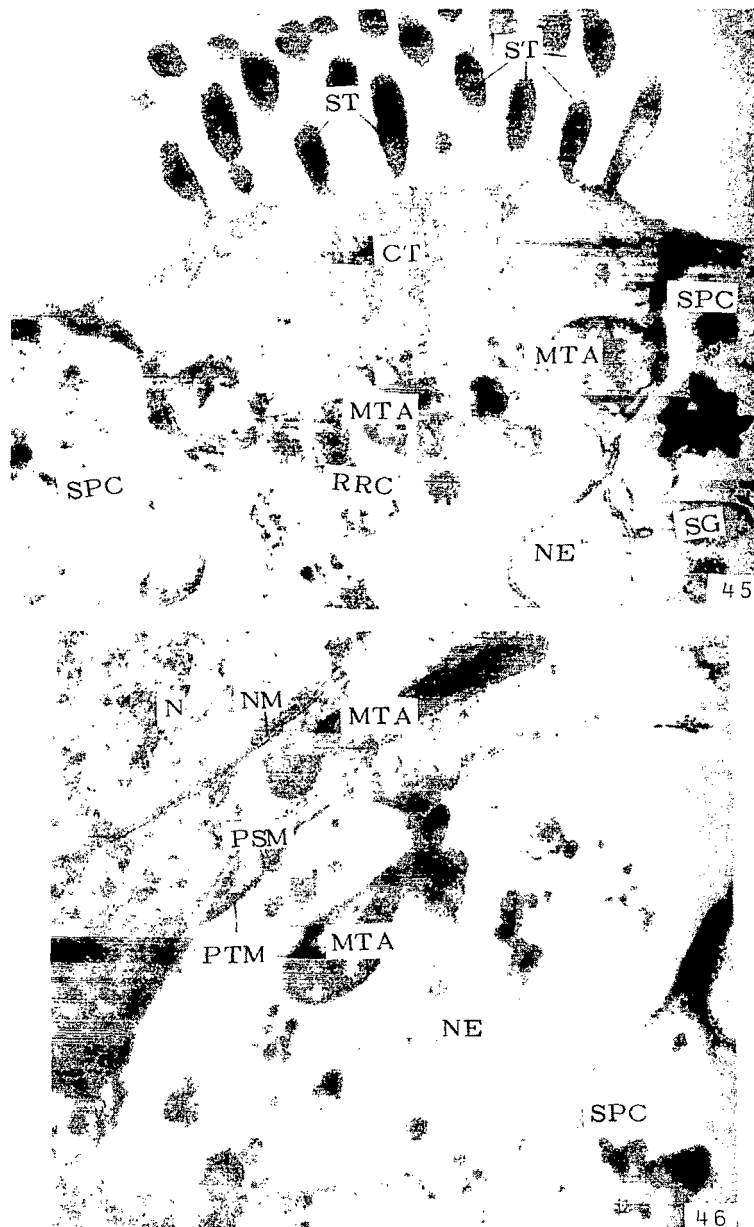


Fig. 45. Summit of a Type I Receptor Cell of the Utricula Macula of a Chick Embryo Before Hatching. Electron Microphotograph, mag. 26,000.

Fig. 46. Base of a Receptor Cell and a Nerve Ending of the Utricula Macula of a Chick Embryo Before Hatching. Electron Microphotograph, mag. 35,000.

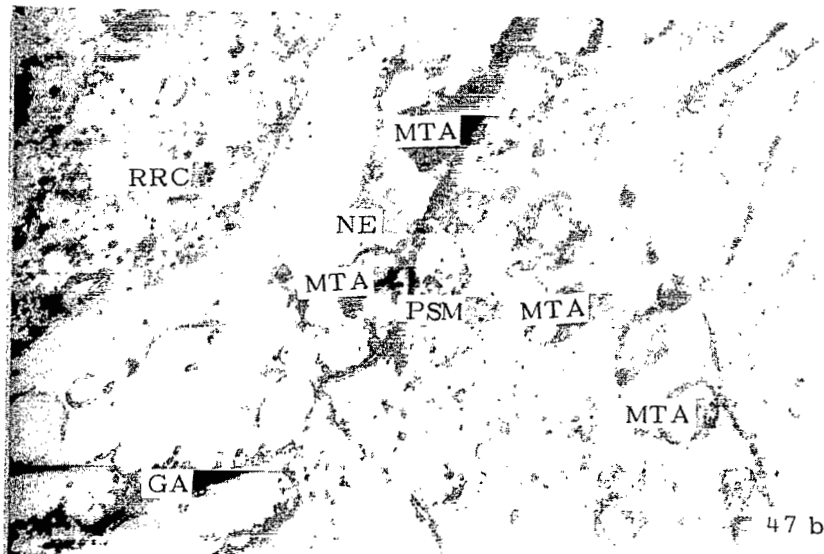
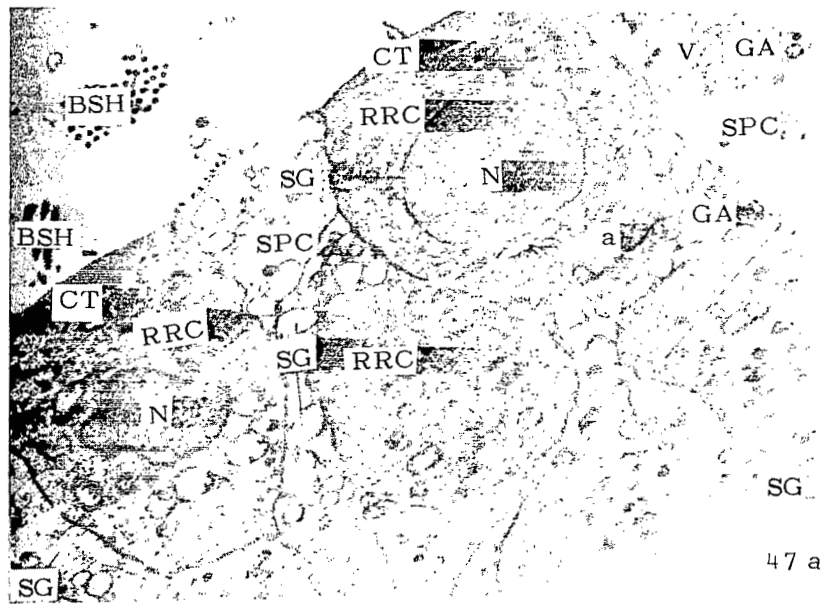


Fig. 47. (a) Upper Portion of the Receptor Epithelium of the Utricula Macula of a Chick 5 Days After Hatching; Electron Microphotograph, mag. 4600; (b) Region a of the Same Section with Magnification $\times 11,400$;

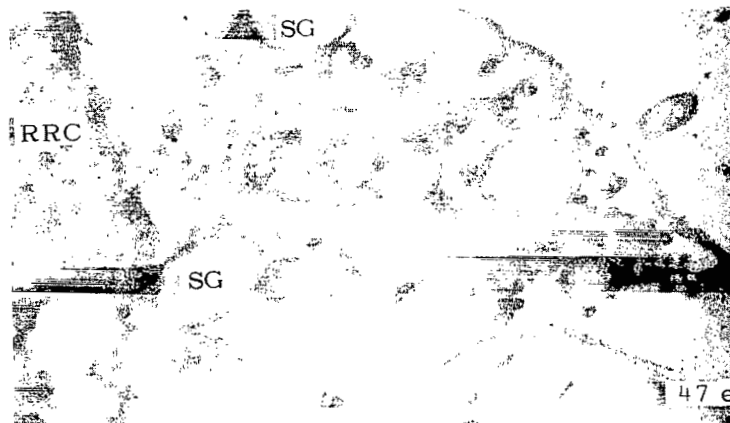
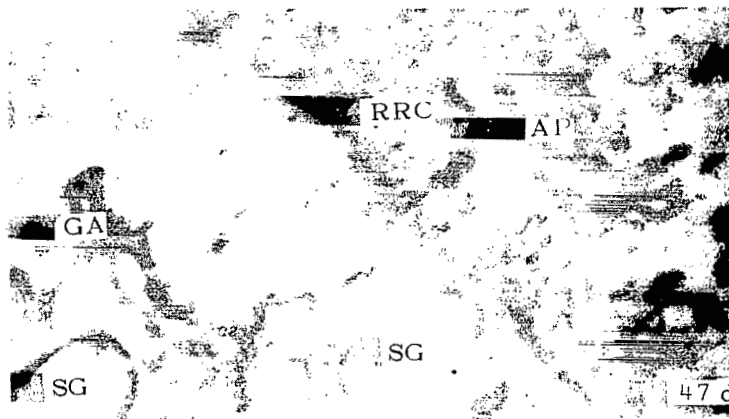
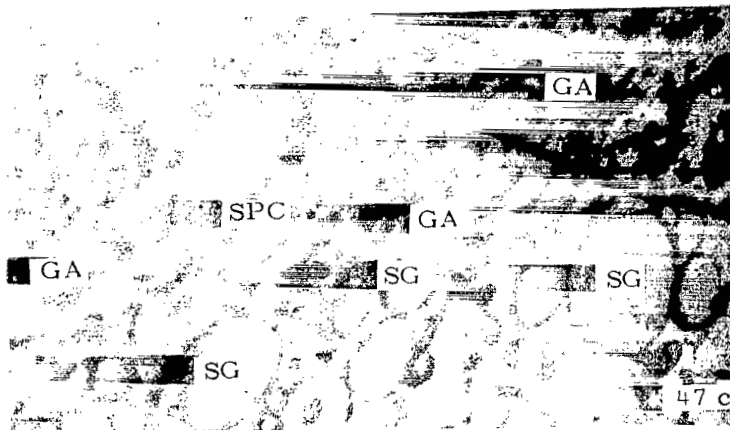


Fig. 47 (continuation). (c) Region b with Magnification 17,800; (d) Region b with Magnification 44,000; (e) Region a with Magnification 40,000.



Fig. 48. Receptor Epithelium of the Utricular Macula of a Chick 9 Days After Hatching. Electron Microphotograph, mag. 10,500.

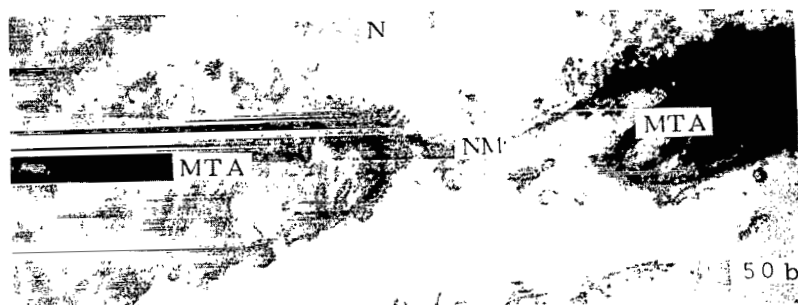
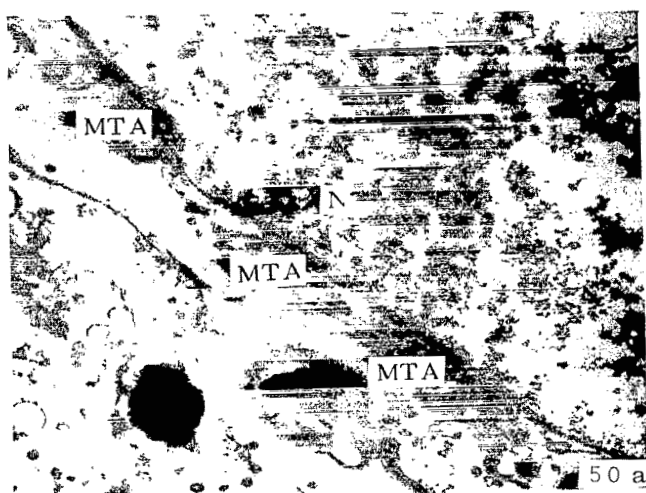


Fig. 49. Part of a Receptor Cell of a Chick 9 Days After Hatching. Attachment of Mitochondria to the Nucleus. Electron Microphotograph, mag. 54,000.

Fig. 50. (a) Attachment of Mitochondria to the Nucleus of a Receptor Cell of a Chick 9 Days After Hatching; Electron Microphotograph, mag. 54,000; (b) The Same with Magnification of 140,000.

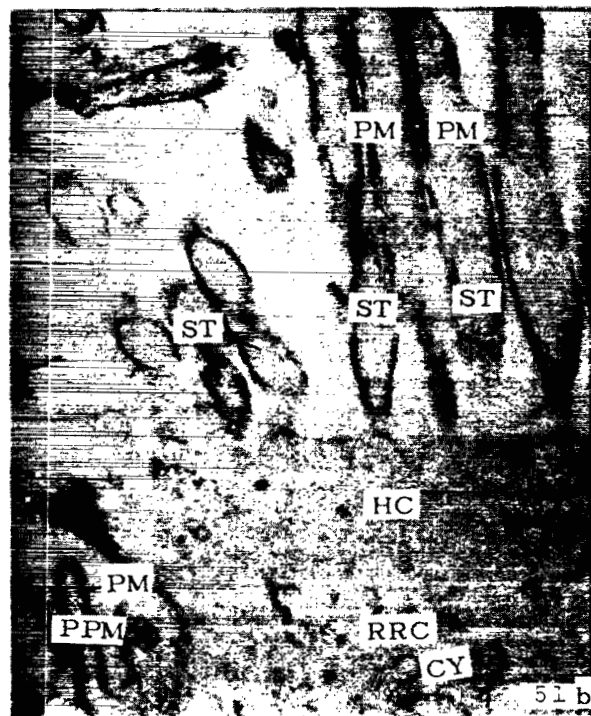


Fig. 51. (a) Upper Portion of the Receptor Epithelium of the Utricular Macula of a Chick 9 Days After Hatching; Electron Microphotograph, mag. 13,200; (b) Summit of the Same Receptor Cell, mag. 88,000.

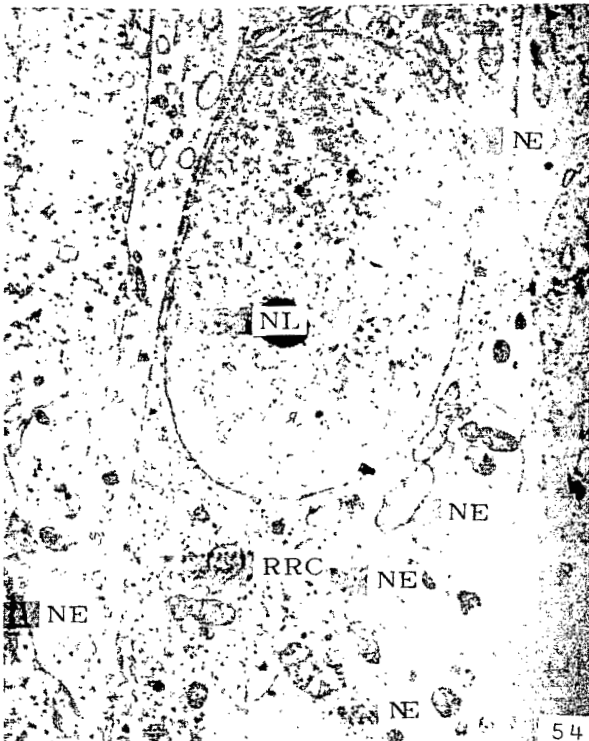
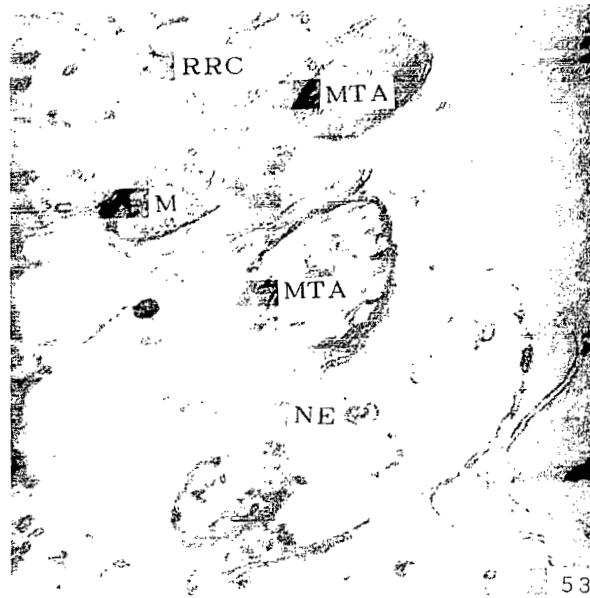
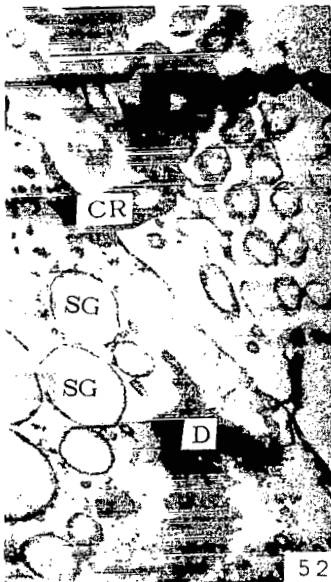


Fig. 52. Cilia Rod on a Supporting Cell of the Receptor Epithelium of the Utricle Macula of a Chick 9 Days After Pecking Out. Electron Microphotograph, mag. 7000.

Fig. 53. The Same Stage as Fig. 52. Nerve Ending. Electron Microphotograph, mag. 52,000.

Fig. 54. Same Stage as Fig. 52. Nerve Ending Around a Receptor Cell in the Utricle Macula. Electron Microphotograph, mag. 3000.

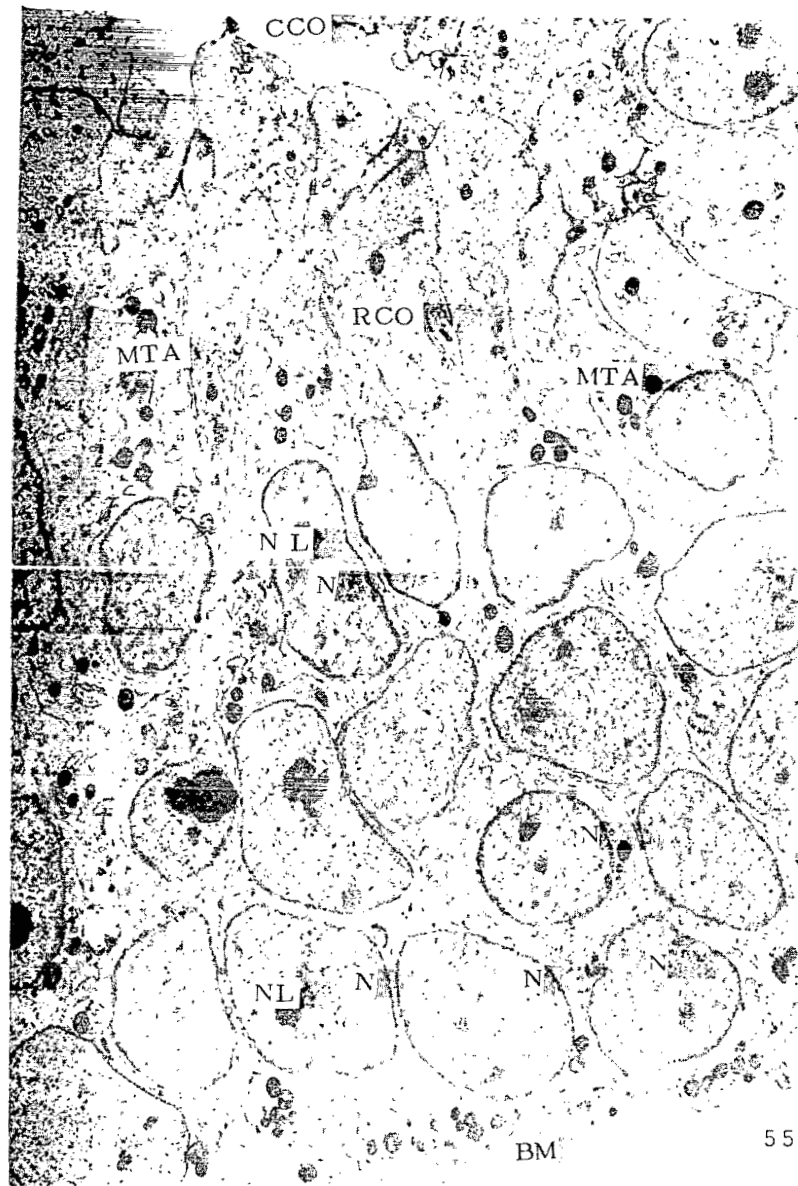


Fig. 55. Receptor Epithelium of Corti's Organ of a Chick Embryo of 7 Days Incubation. Electron Microphotograph, mag. 3000.

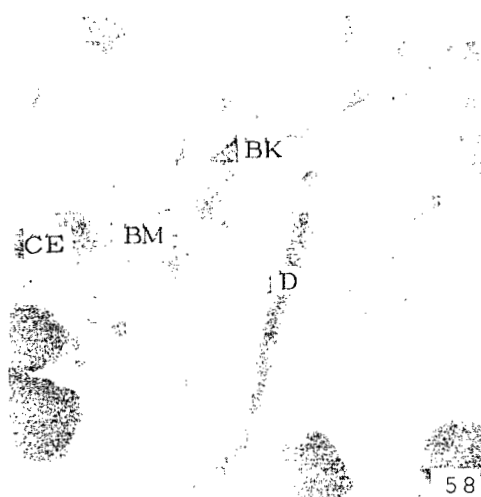
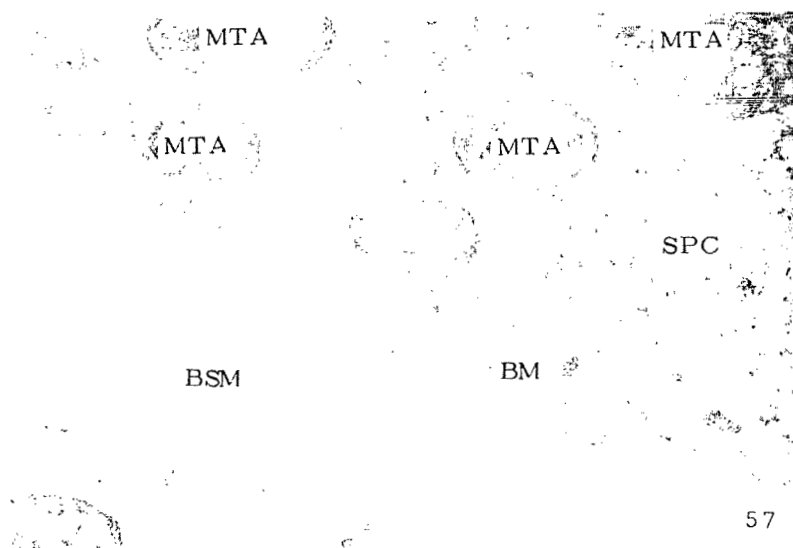


Fig. 56. A Developing Kinocilium at the Summit of a Cell of Corti's Organ of a Chick Embryo of 7 Days Incubation. Electron Microphotograph, mag. 60,000.

Fig. 57. Accumulation of Mitochondria Around the Basal Membrane of the Receptor Epithelium of Corti's Organ of a Chick Embryo of 7 Days Incubation. Electron Microphotograph, mag. 15,800.

Fig. 58. A Developing Kinocilium on the Receptor Epithelium of Corti's Organ of a Chick Embryo of 7 Days Incubation. Electron Microphotograph, mag. 12,000.

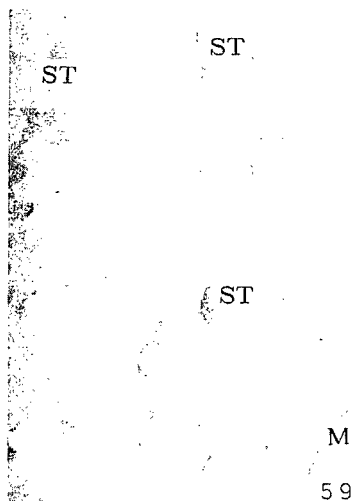
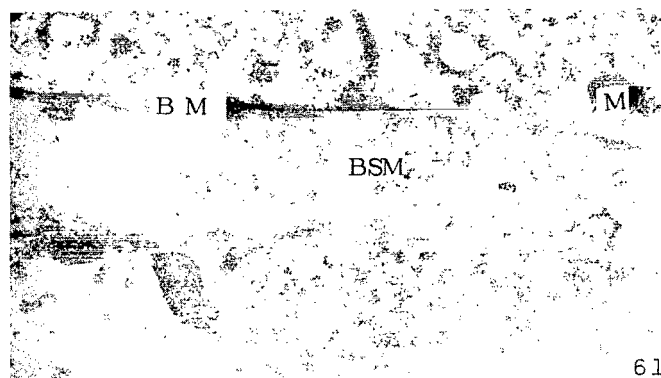
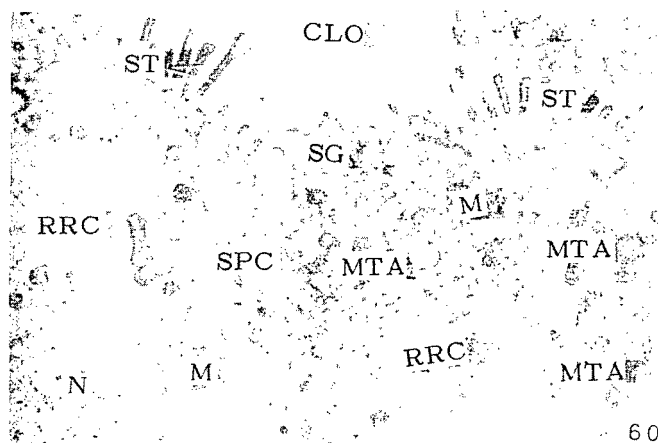


Fig. 59. Development of Stereocilia in the Receptor Epithelium of Corti's Organ of a Chick Embryo of 7 Days Incubation. Electron Microphotograph, mag. 26,000.

Figs. 60 and 61. Upper Portion of the Receptor Epithelium of a Chick Embryo of 11 Days Incubation. Electron Microphotograph, mag. 11,000 (Fig. 60) and 25,000 (Fig. 61).



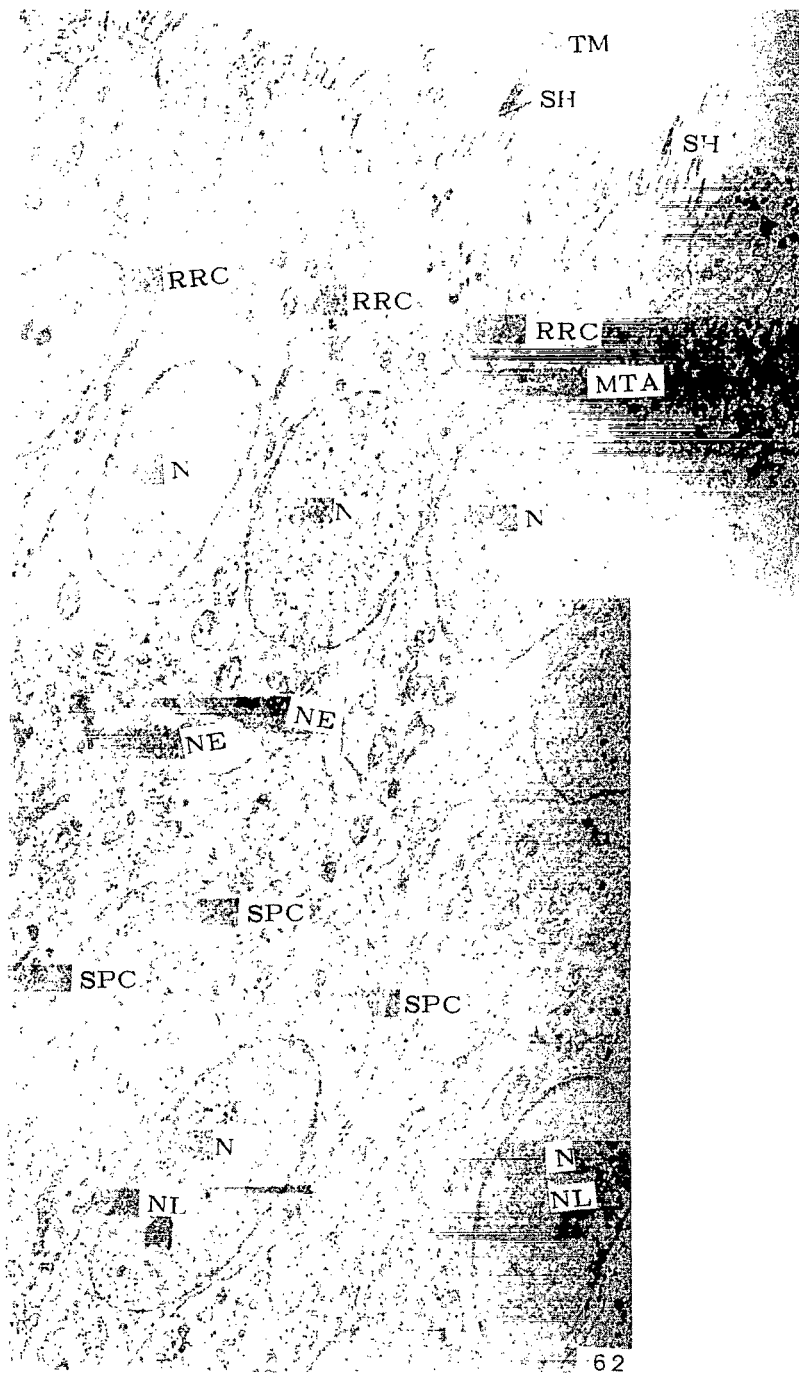


Fig. 62. Receptor Epithelium of Corti's Organ of a Chick Embryo of 15 Days Incubation. Electron Microphotograph, mag. 5600.

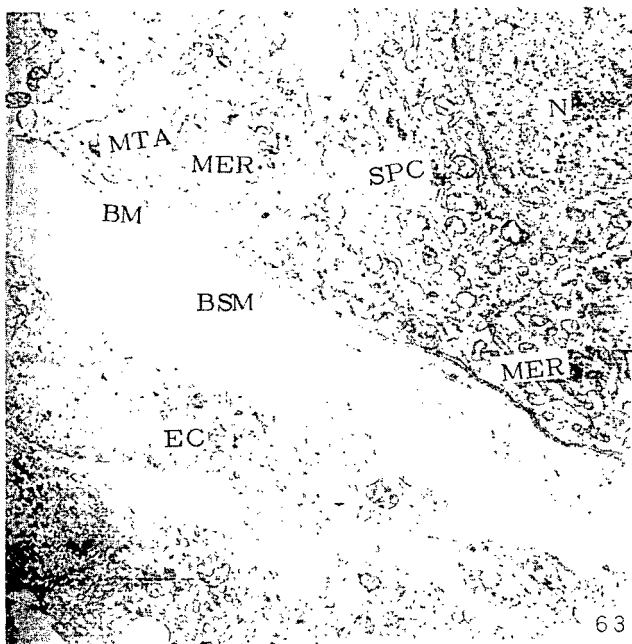


Fig. 63. The Same Stage as on Fig. 62. Base of the Receptor Epithelium of a Chick Embryo of 15 Days Incubation. Basilar Membrane. Electron Microphotograph, mag. 14,000.



Fig. 64. Position of the Kinocilium on a Receptor Cell of Corti's Organ of a Chick Embryo of 15 Days Incubation. Electron Microphotograph, mag. 21,000.

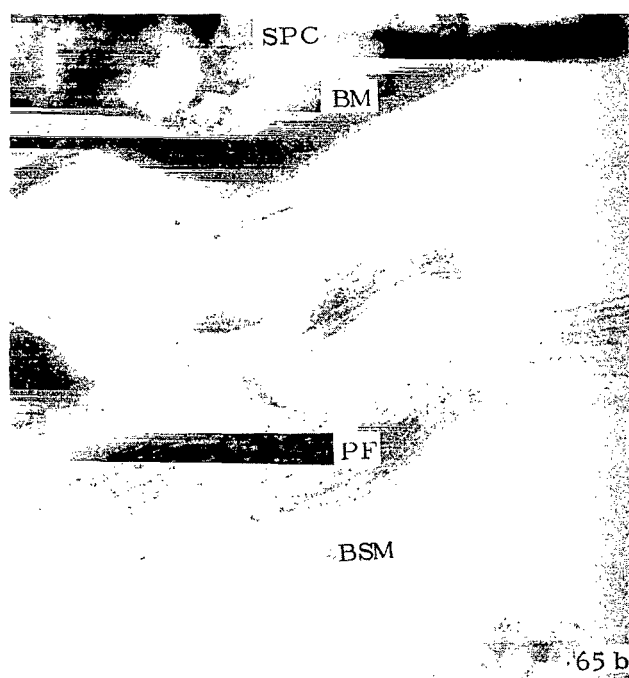
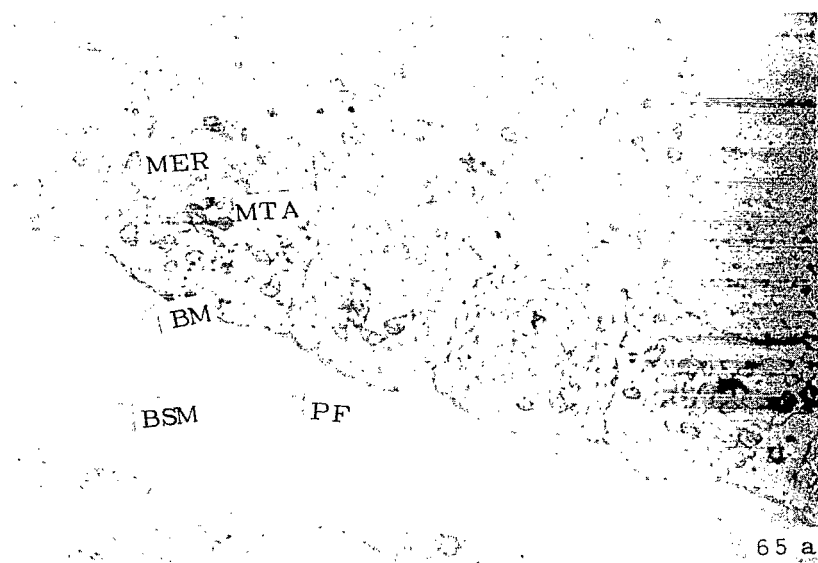


Fig. 65. Corti's Organ. Developing Basilar Membrane of a Chick Embryo of 15 Days Incubation. Electron Microphotograph: (a) with Magnification 5800; (b) with Magnification 200,000.

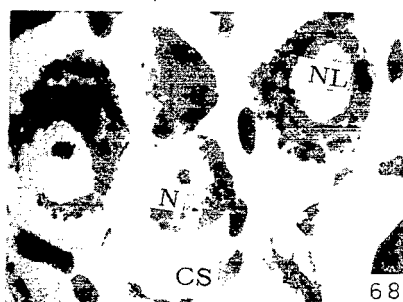
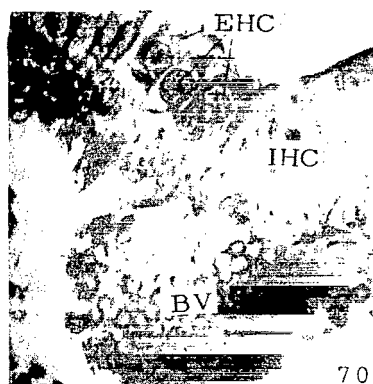
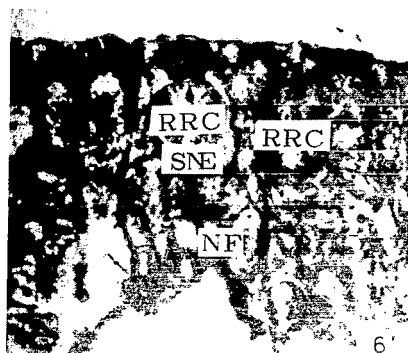
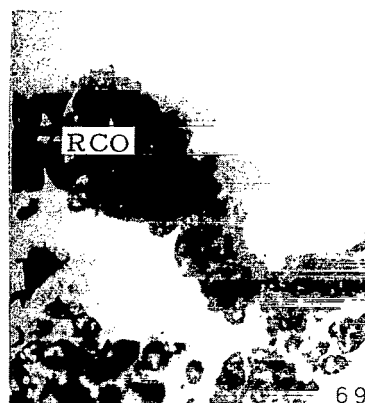
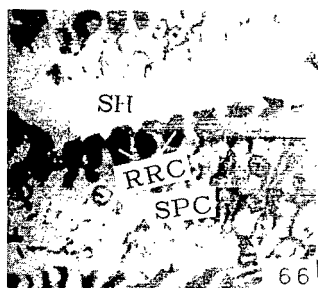


Fig. 66. Receptor Epithelium of Corti's Organ of a Chick 2 Days After Hatching Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. hom. $\times 6$.

Fig. 67. Utricular Macula of a Rabbit Embryo of 28 Days of Intra-uterine Life. Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. $\times 10$.

Fig. 68. Vestibular Ganglion of a Rabbit 1 Day After Birth; Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. $\times 10$.

Fig. 69. Corti's Organ of the Superior Helix of the Cochlea of a Rabbit Embryo with 17 Days of Intrauterine Life. Treated for Nucleic Acids According to Kurnik; imm. ob. $\times 60$, oc. hom. 6.

Fig. 70. Corti's Organ of the Basal Helix of the Cochlea of a New-born Kitten. Treated for Total Protein According to Danielli; imm. ob. $\times 60$, oc. hom. 6.

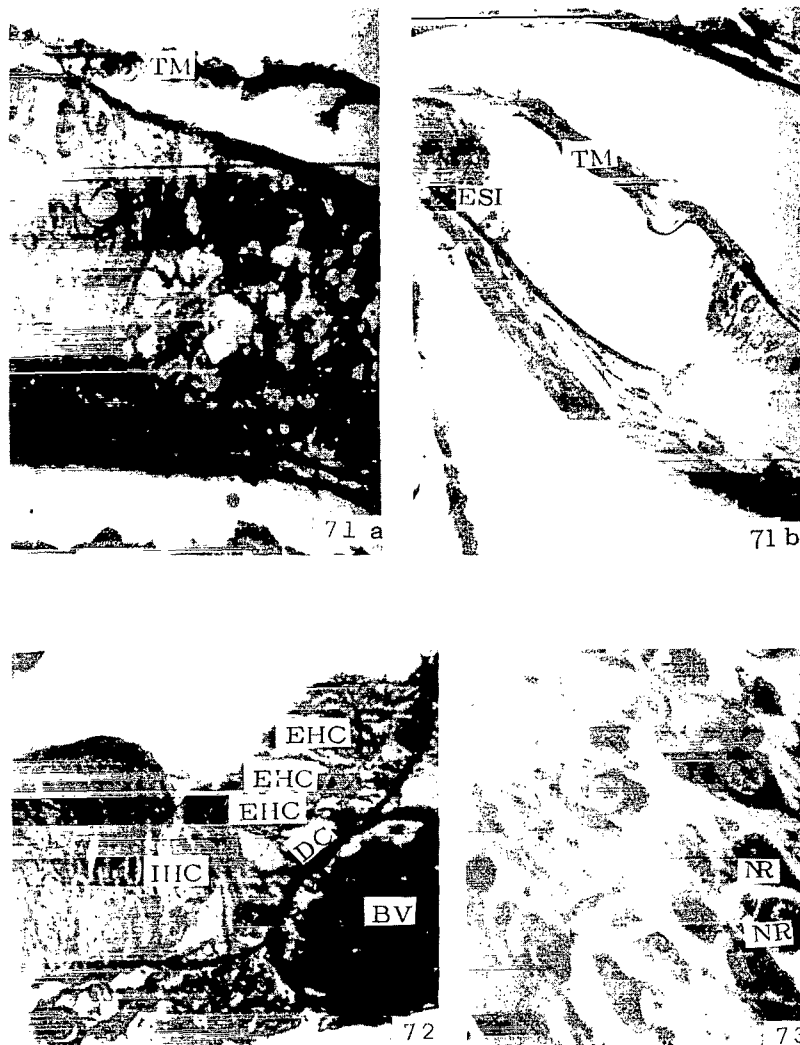


Fig. 71. (a) Corti's Organ of the Basal Helix of the Cochlea of a Kitten 2-1/2 Days After Birth. Formation of the Spiral Incisure. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. hom. 6; (b) Formation of the Spiral Incisure of a Kitten 10 Days After Birth. Treated for Total Protein; imm. ob. $\times 60$, oc. hom. 6.

Fig. 72. Corti's Organ of the Second Helix of the Cochlea of a Kitten 10 Days After Birth. Treated for Thiol Groups According to Yakovlev and Nistratova; imm. ob. $\times 60$, oc. hom. 6.

Fig. 73. The Spiral Ganglion of the Second Helix of the Cochlea of a Kitten 10 Days After Birth. Treated for Carboxyl Groups According to Barrnett and Seligman; imm. ob. $\times 60$, oc. hom. 6.

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